Title: Persistent Methicillin Resistant Staphylococcus Aureus Eradication Protocol (PMEP)

NCT01594827

PI: Michael Boyle, MD

Date: November 25, 2015

Clinical Research Protocol

TITLE: PERSISTENT MRSA ERADICATION PROTOCOL (PMEP)

Protocol Number:	PMEP 3.8
Version Date:	November 25, 2015
Sponsor:	Cystic Fibrosis Foundation 6931 Arlington Road, 2nd floor Bethesda, MD 20814
Principal Investigator:	Name: Michael Boyle, MD 1830 E. Monument Street, 5th floor Baltimore MD, 21205 Telephone: 410-502-7042; Fax: 410-502-7048 E-mail: mboyle@jhmi.edu
Participating Centers:	1. Johns Hopkins Hospital, 1800 Orleans Street , Baltimore, MD, 21287 2. University Hospitals/Case Medical Center, 11100 Euclid Avenue, Cleveland, Ohio 44106
DSMB:	The Cystic Fibrosis Foundation Therapeutics Data and Safety Monitoring Board Chairman: Dr. Wayne Morgan Telephone: (520) 626-6754; Fax: (520) 626-9465 Email: wmorgan@arc.arizona.edu
Coordinating Center:	CFFT Cystic Fibrosis Therapeutics Development Network Coordinating Center, Seattle, WA 98101
Participating Laboratories	Johns Hopkins Medical Laboratories, 1800 Orleans Street, Zayed B 1065, Baltimore, MD 21287 University Hospital Case Medical Center Laboratories, 11100 Euclid Avenue, Wearn Building, Room 611, Cleveland, OH 44106 Seattle Children's Hospital, Clinical Microbiology Lab, MS A-6901, 4800 Sand Point Way NE, Seattle, WA 98105 Case Western Reserve University, Dept. of Pediatrics, Bonfield Lab, 10900 Euclid Avenue, BRB 8th Floor, LC 4948, Room 822, Cleveland, Ohio 44106

Approval:

Michael Boyle, MD _____ *Date*

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Cystic Fibrosis Foundation with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: PMEP 3.8

Protocol Title: **PERSISTENT MRSA ERADICATION PROTOCOL (PMEP)**

Protocol Date: 11/25/2015

_____ _____

Investigator Signature *Date*

_____

Print Name and Title

This confidential information is provided for the exclusive use of investigators of this trial and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information. These restrictions on disclosure will apply equally to all future oral or written information, which is designated as "privileged" or "confidential".

TABLE OF CONTENTS

List of Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFF	Cystic Fibrosis Foundation
CFQ-R	Cystic Fibrosis Quality of Life Questionnaire - Revised
CFTR	Cystic fibrosis transmembrane conductance regulator
CF	cystic fibrosis
CRF	case report form
CRP	C-reactive protein
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume over one second
FEV_1%	Forced expiratory volume over one second percentage of predicted
FVC	Forced vital capacity
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
IEC	Independent Ethics Committee
IL-8	Interleukin-8
IRB	Institutional Review Board
LDH	lactate dehydrogenase
mEq	milliequivalent
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
PI	Principal Investigator
PK	pharmacokinetic
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
TDN-CC	Therapeutics Development Network- Coordinating Center
TMP/SMX	Trimethoprim / Sulfamethoxazole (Bactrim)
VISA	Vancomycin-insensitive *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*

PROTOCOL SYNOPSIS

TITLE	**Persistent MRSA Eradication Protocol (PMEP)**
SPONSOR	Cystic Fibrosis Foundation
NUMBER OF SITES	2 (University Hospitals Case Medical Center and Johns Hopkins University)
RATIONALE	The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) respiratory infection in CF has increased dramatically over the last decade. Epidemiologic evidence suggests that persistent infection with MRSA may result in an increased rate of decline in FEV_1 and shortened survival. Currently there are no conclusive studies demonstrating an effective aggressive treatment protocol for persistent MRSA respiratory infection in CF. Data demonstrating an effective and safe method of clearing persistent MRSA infection are needed before potentially embarking on a national interventional trial.
STUDY DESIGN	This is a two-center (Case and Johns Hopkins), randomized and stratified on center and FEV_1% predicted (FEV_1% ≤ 60% and FEV_1% > 60%) double-blind, comparator-controlled, parallel-group study with 1:1 assignment to either vancomycin for inhalation (250 mg twice a day) or taste matched placebo for 28 days in individuals with cystic fibrosis. Patients will be followed for 3 additional months. In addition, both groups will receive oral rifampin, a second oral antibiotic (TMP-SMX or doxycycline, protocol determined), mupirocin intranasal cream and chlorhexidine body washes. 40 patients with persistent respiratory tract MRSA infection will be enrolled: 20 will be randomized to vancomycin for inhalation and 20 to a taste-matched placebo.
PRIMARY OBJECTIVE	To evaluate the safety and efficacy of a 28-day course of vancomycin for inhalation, 250 mg twice a day, (in combination with oral antibiotics) in eliminating MRSA from the respiratory tract of individuals with CF and persistent MRSA infection.

SECONDARY OBJECTIVES	To evaluate the efficacy of a 28-day course of vancomycin for inhalation (250 mg twice a day) in addition to oral antibiotics in individuals with CF and persistent MRSA infection on: • FEV_1% predicted • CFQ-R (respiratory) • Subsequent exacerbations (using standardized exacerbation scoring criteria-see appendix 3). • MRSA colony forming units To evaluate the safety of a 28-day course of vancomycin for inhalation (250 mg twice a day) in addition to oral antibiotics, monitor for increasing incidence of gram negative pathogens, antimicrobial resistance to rifampin, trimethoprim/sulfamethoxazole (TMP/SMX), doxycycline, and vancomycin; vancomycin sputum and serum levels. To evaluate the change in strains of MRSA (PFGE analysis), emergence of small colony variants of MRSA, and changes in sputum cell count and sputum cytokine profiles
NUMBER OF SUBJECTS	Forty (40)

SUBJECT SELECTION CRITERIA	Inclusion Criteria: 1. Male or female ≥ 12 years of age. 2. Confirmed diagnosis of CF based on the following criteria: positive sweat chloride > 60 mEq/liter (by pilocarpine iontophoresis) and/or a genotype with two identifiable mutations consistent with CF or abnormal NPD, and one or more clinical features consistent with the CF phenotype. 3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study. 4. Two positive MRSA respiratory cultures in the last two years at least six months apart, plus a positive MRSA respiratory culture at Screening Visit and Run-in (Day -14) Visit. 5. At least 50% of respiratory cultures from the time of the first MRSA culture (in the last two years) have been positive for MRSA. 6. FEV1 ≥40% of predicted normal for age, gender, and height at Screening, for subjects 18 years of age and older. 7. FEV1 ≥60% of predicted normal for age, gender, and height at Screening, for subjects 12-17 years old. 8. Females of childbearing potential must agree to practice one highly effective method of birth control, including abstinence. Note: highly effective methods of birth control are those, alone or in combination, that result in a failure rate less than 1% per year when used consistently and correctly. Female patients who utilize hormonal contraceptives as a birth control method must have used the same method for at least 3 months before study dosing. If the patient is using a hormonal form of contraception and is receiving rifampin, patients will be required to also use barrier contraceptives as rifampin can affect the reliability of hormone therapy. Barrier contraceptives such as male condom or diaphragm are acceptable if used in combination with spermicides. Exclusion Criteria: 1. An acute upper or lower respiratory infection, pulmonary exacerbation, or change in routine therapy (including antibiotics) for pulmonary disease within 42 days of the Day 1 Visit (2 weeks prior to screening visit). 2. Individuals on continuous inhaled antibiotics without interruption who are not willing to substitute vancomycin or placebo for their scheduled inhaled antibiotic during days 0-28 of the study (every other month inhaled antibiotics are acceptable).

	3. Use of oral or inhaled anti-MRSA drugs within two weeks of the Screening Visit. 4. History of intolerance to inhaled vancomycin or inhaled albuterol. 5. History of intolerance to both TMP/SMX and doxycycline. 6. Resistance to both TMP/SMX and doxycycline at Screening. 7. Resistance to vancomycin at Screening. 8. Abnormal renal function, defined as creatinine clearance <50 mL/min using the Cockcroft-Gault equation for adults or Schwartz equation in children, at Screening. 9. Abnormal liver function, defined as ≥3x upper limit of normal (ULN), of serum aspartate transaminase (AST) or serum alanine transaminase (ALT), or known cirrhosis at the time of Screening. 10. Serum hematology or chemistry screening results which in the judgment of the investigator would interfere with completion of the study. 11. History of or listed for solid organ or hematological transplantation. 12. History of sputum culture with non-tuberculous *Mycobacteria* in the last 6 months. 13. History of sputum culture with *Burkholderia Cepacia* in the last year. 14. Planned continuous use of soft contact lenses while taking rifampin and no access to glasses. 15. Current use of oral corticosteroids in doses exceeding the equivalent of 10 mg prednisone a day or 20 mg prednisone every other day. 16. Administration of any investigational drug or device within 28 days of Screening or within 6 half-lives of the investigational drug (whichever is longer). 17. Patients on inhaled antibiotics who have not been on the same regimen for the 4 months prior to screening 18. Female patients of childbearing potential who are pregnant or lactating, or plan on becoming pregnant 19. Any serious or active medical or psychiatric illness, which in the opinion of the investigator, would interfere with patient treatment, assessment, or adherence to the protocol.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION	Days 1-28: 1) Nebulized Vancomycin a. 250 mg nebulized two times a day for 28 days in 5cc sterile water. Patients will use a Pari Sprint nebulizer and Pari Vios compressor as the delivery system. 2) Oral Rifampin a. >45 kg: 600 mg by mouth daily b. 35-45 kg : 450 mg by mouth daily c. 25-34.9 kg: 300 mg by mouth daily 3) Protocol guided therapy a. Oral trimethoprim/sulfamethoxazole (DS-160/800) i. >45 kg: two Double Strength (DS) tablets twice a day by mouth (320/1600) ii. 25-45 kg: one DS tablet twice a day by mouth (160/800) b. If sulfa intolerant or TMP/SMX resistant, use instead oral doxycycline i. >45 kg: 100 mg by mouth twice a day ii. 35-45 kg : 75 mg by mouth twice a day iii. 25-34.9 kg: 50 mg by mouth twice a day 4) Mupirocin 2% intranasal ointment: ½ of single use tube applied into each nostril twice a day x 5 days 5) Hibiclens 15 cc liquid skin cleanser packets (4% chlorhexidine gluconate): use three packets once weekly for four weeks in the shower from the neck to toes, with attention on the axilla, groin, and buttocks. 6) Environmental: While wearing gloves, wipe down high touch surfaces with Sani-Cloth Alcohol Free Germicidal wipes (provided) and wash all bed linens and towels in hot water weekly for the first three weeks.
CONTROL PRODUCT, DOSE AND MODE OF ADMINISTRATION	Days 1-28 1) Nebulized Placebo a. Volume (5cc) and taste (quinine 0.1mg/mL) matched nebulized placebo (sterile water) for 28 days. 2) Oral Rifampin a. >45 kg: 600 mg by mouth daily b. 35-45 kg : 450 mg by mouth daily c. 25-34.9 kg: 300 mg by mouth daily 3) Protocol guided therapy a. Oral trimethoprim/sulfamethoxazole (DS-160/800) i. >45 kg: two DS tablets twice a day by mouth (320/1600) ii. 25-45 kg: one DS tablet twice a day by mouth (160/800)

	b. If sulfa intolerant or TMP/SMX Resistant, use instead oral doxycycline i. >45 kg: 100 mg by mouth twice a day ii. 35-45 kg : 75 mg by mouth twice a day iii. 25-34.9 kg: 50 mg by mouth twice a day 4) Mupirocin 2% intranasal ointment: half of single use tube applied into each nostril twice a day X 5 days. 5) Hibiclens 15cc liquid skin cleanser packets (4% chlorhexidine gluconate): use three packets once weekly for four weeks in the shower from the neck to toes, with attention on the axilla, groin, and buttocks. 6) Environmental: While wearing gloves, wipe down high touch surfaces with Sani-Cloth Alcohol Free Germicidal wipes (provided) and wash all bed linens and towels in hot water weekly for the first three weeks.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 155 days **Screening/Run In:** up to 30 days **Treatment:** 28 days **Primary Endpoint:** Day 58 **Follow-up:** 3 months after completion of 28-day treatment period The total duration of the study is expected to be 48 months: 45 months for subject recruitment and 3 months for final subject follow-up.
CONCOMMITANT MEDICATIONS	Allowed: All routine chronic CF medications except non-study anti-MRSA antibiotics (unless medically indicated). Inhaled anti-pseudomonal antibiotics will be permitted except during the 28 days study drug treatment period. Prohibited: Regular use of loop diuretics during days 1-28, inhaled antibiotics other than study drug on days 1-28, IV antibiotics from day -42 to day 28 (unless medically indicated).
EFFICACY EVALUATIONS	Culture (quantitative and qualitative) and sensitivity; FEV_1% predicted; CFQ-R (pulmonary); Exacerbations (standardized definition in appendix 3)
PRIMARY ENDPOINT	Percentage of patients MRSA free by induced sputum respiratory tract culture one month after completion of four-week eradication protocol (Day 58) in intervention arm vs. control arm
SECONDARY ENDPOINTS	• Percentage of patients MRSA free by induced sputum respiratory tract culture at Visit 4 (Day 29) and Visit 6 (Day 118) after completion of 28-day treatment period. • Change in FEV_1% predicted from baseline and screening to Visit 4 (Day 29), Visit 5 (Day 58), and Visit 6 (Day 118). • Time to first pulmonary exacerbation (standard definition in appendix 3). • Time to first anti-MRSA antibiotics (after treatment period)

	• Total number of pulmonary exacerbations at Visit 5 (Day 58) and Visit 6 (Day 118). • Change in patient reported CFQ-R (respiratory) from baseline at Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (day 58), and Visit 6 (Day 118). • Number of patients with newly developed MRSA resistance to vancomycin, TMP/SMX, doxycycline, and/or rifampin.
OTHER EVALUATIONS	• Serum vancomycin peak level 60 minutes after completion of first dose of inhaled vancomycin on Visit 1 • Trough serum vancomycin level to be drawn prior to nebulization of vancomycin on Day 14 (two weeks into nebulized vancomycin). • Sputum vancomycin level at Visit 1, 5 minutes after nebulizing vancomycin. • Change in minimum inhibitory concentration (MIC) of vancomycin, rifampin, TMP/SMX, and tetracycline for MRSA. • Strain analysis of MRSA for the epidemiologic study of Hospital Acquired (HA) and Community Acquired (CA) MRSA and the genetic relatedness of MRSA strains • Skin (axillary) and nares nasal swabs for MRSA • Emergence of small colony variants of MRSA • Sensitivity analysis based on time infected with MRSA
SAFETY EVALUATIONS	Chemistry panel, AST, ALT, alkaline phosphatase, and CBC with differential, at Screening (Day -28), Visit 1 (Day 1), Visit 3 (Day 14), Visit 4 (Day 29) and Visit 5 (Day 58) and Early Withdrawal. Incidence of adverse events. Continuous oxygen saturation checked throughout nebulized vancomycin dose at Visit 1 (Day 1), Visit 2 (Day 2-7), and Visit 3 (Day 14). Spirometry checked immediately after and 15 minutes (+/-10 mins.) after nebulized vancomycin dose is completed at Visit 1(Day 1) and Visit 2 (Day 2-7). Serum vancomycin levels. If a trough level at Day 14 is found to be greater than 8 micrograms/mL we will terminate inhaled study drug. Hand washing with chlorhexidine body wash at Run In Visit (-14 days) to assess for skin sensitivity.
SAFETY MONITORING	Dr. Wayne Morgan, chair of the CFF DSMB, has agreed to chair a data monitoring committee composed of a biostatistician and another physician. The DSMB will review the data after the first 16 adults (≥ age 18) have been enrolled (8 in each group). If there are no safety issues, then we will start enrolling individuals 12-17 years of age as well. The DSMB will perform an interim look after the first 8 pediatric patients have been dosed and/or six months following IRB approvals to enroll, whichever comes first. The DSMB will receive monthly reports including enrollment for both

	pediatric and adult groups, screen failures or study withdrawals, and FEV1 follow up data after administration of study drug.
PRIMARY ANALYSIS PLAN (STATISTICS)	Our primary analysis will compare the proportion of CF patients who have MRSA eradicated from their respiratory tract in the intervention group to the standard care group one month after completion of the treatment period (Visit 5 at day 58) using Pearson's χ^2 test. A secondary analysis will be performed comparing proportions of MRSA eradication at Visit 4 (day 29) and Visit 6 (day 118) after completion of 28-day treatment period Student's t-test will be performed to compare the change in FEV_1% predicted from baseline and screening between intervention and control groups at Visit 4 (Day 29), Visit 5 (Day 58) and Visit 6 (Day 118). We will also use the General Estimating Equations (GEE) framework to longitudinally analyze the change in lung function over 118 days. The respiratory domain of CFQ-R questionnaire will be expressed as mean ± standard deviation and statistical significance assessed between the groups using a 2-sample t-test from baseline (Visit 1) to Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (Day 58) and Visit 6 (Day 118). A Kaplan-Meier plot will be used to graphically display estimates of the survivor function for the proportion of patients who do not have an exacerbation through day 118. Hazard ratios and 95% CI due to treatment will be calculated using a Cox proportional hazards regression model. Pre-specified potential variables that may be adjusted for include gender, season of enrollment, and center.
RATIONALE FOR NUMBER OF SUBJECTS	Enrollment of 40 participants (20 intervention, 20 control) will give us 92% power to detect a 55% absolute increase in percent of patients with MRSA not detected by induced sputum microbiology (75% in the intervention arm vs. 20% resolution in the control arm, two tail alpha=0.05)

1 BACKGROUND

1.1 Overview

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder in the Caucasian population[1]. With improvements in care, the average age of individuals living with CF continues to increase, and the median survival age is approximately 37 years[2]. One consequence of improving survival is the emergence of pulmonary infections with new and resistant pathogens. These infections are of importance because they may lead to respiratory failure, which continues to be the leading cause of mortality in individuals with CF. Methicillin-resistant Staphylococcus aureus (MRSA) is a particularly important emerging pathogen in CF. The prevalence of infection with methicillin-resistant Staphylococcus aureus (MRSA) in the CF community has increased from 4% in 1999 to 23.7% in 2009[3].

Recently, two very large CF observational studies utilizing the CF Foundation's National Patient Registry database have demonstrated that infection with MRSA is associated with worse clinical outcomes[4;5]. The first, published in 2008 by Dasenbrook et al. demonstrated that persistent respiratory infection with MRSA is associated with a more rapid decline in lung function as measured by FEV_1. The second, also published by Dasenbrook et al. in JAMA in 2010, demonstrated an association between persistent MRSA infection and increased mortality, even after adjustment for severity of lung disease.

Given the striking increase in both prevalence of MRSA infection in CF and evidence of a detrimental effect of MRSA on CF clinical outcomes, there has been growing interest in treatment protocols designed to treat and/or eradicate MRSA CF respiratory infection.

1.2 Previous Studies of Treatment of MRSA in CF

1.2.1 Non-clinical Studies

There have been few non-clinical investigations of MRSA infections in CF. One study in mice demonstrated that CFTR-deficient mice were much more susceptible to chronic MRSA nasal infection than normal controls[6]. Another study in rats demonstrated that nebulized vancomycin achieved dramatically higher drug levels in the airway surface liquid layer than systemically administered vancomycin[7]. In this same study, vancomycin was not detectable systemically in the rats after nebulization, suggesting that minimal amounts of vancomycin crossed the alveolar-capillary membrane when administered via the respiratory route. The authors concluded that nebulized vancomycin was likely to be associated with a very low risk of ototoxicity or nephrotoxicity in people because of the minimal observed systemic absorption.

1.2.2 Clinical Studies

There have been several small clinical studies in CF assessing MRSA treatment regimens and their effectiveness in eradicating MRSA from the respiratory tract. These studies have been limited however by small study populations, lack of control groups, single-center retrospective design, variable follow-up, and failure to distinguish incident vs. persistent MRSA infection[8-11]. Doe and colleagues have reported the largest experience to date with a retrospective review of 37 patients at their Adult CF center in Manchester, UK [11]. Although many different eradication regimens were used, most of the patients in their cohort (average age 25.6 years, mean FEV_1 2.2 L, and 75% with *Pseudomonas aeruginosa*) were treated with a combination of two oral antibiotics (rifampicin, fusidic acid, or trimethoprim) and nebulized vancomycin (200 mg four times a day for five days). They reported that this resulted in eradication of MRSA in 81% of the participants at 6 months. There was no distinction made between those with incident MRSA and those with persistent MRSA infection in the study analysis, although they report that approximately 38% of those included had had multiple positive MRSA cultures. There is one small study by Garske and coworkers which focused on treatment of CF adults with persistent MRSA[8] (perhaps similar to our study population). They enrolled seven CF adults with persistent MRSA (average FEV_1 36% predicted, all with chronic PA, 6/7 with previous IV MRSA therapy); 5 of the 7 patients (71%) were culture-negative six months after completing a six month treatment regimen of oral fusidic acid and rifampin[8]. Most other studies have focused on patients with incident MRSA infection rather than persistent infection. Macfarlane and co-workers reported on 17 CF patients with incident MRSA treated with

oral and IV antibiotics. Utilizing a protocol which started with oral rifampicin and fusidic acid, they achieved a 94% eradication rate at 12 months[9]. A study by Solis and coworkers of 15 CF children treated with five-days of oral and nebulized vancomycin was associated with a 55% eradication rate [10].

1.3 Previous Experience with Nebulized Vancomycin

There have been numerous reports of the clinical use of nebulized vancomycin in both CF and non-CF populations, all of which have demonstrated it to be well-tolerated and efficacious. The doses reported in those aged >12 years of age have included 125 mg twice a day, 250 mg four times a day, and 500 mg twice a day[12]. In the largest study to date, 51 non-CF patients received 125 mg of nebulized vancomycin twice a day for an average of 14.7 days to eradicate respiratory MRSA[13]. Eradication success rate was 84.3%. The authors reported that there were no adverse events associated with inhalation of vancomycin in the 51 participants. Also of importance, vancomycin was not detectable by serum levels two hours after inhalation of the 125 mg of vancomycin. In a study by Doe and coworkers, nebulized vancomycin 200 mg four times a day for 5 days was administered to 18 CF patients[11]. Three experienced chest tightness, but all episodes were graded as mild and none required discontinuation of the drug. Maiz and colleagues also reported their experience with a 10 year-old CF patient treated with nebulized vancomycin 250 mg two times a day for 17 continuous months without adverse events or antibiotic resistance[14]. There was no increase in cough or wheezing during vancomycin administration. The patient had negative fecal cultures for VRE and despite repeatedly checking, there was no development of VISA. The authors also reported that creatinine was normal throughout study and the patient did not develop ototoxicity. Hayes and colleagues reported treating a post-transplant CF patient with refractory MRSA with inhaled vancomycin 250 mg twice a day for six months[15]. The patient had undetectable serum vancomycin levels at 2 hours and despite taking numerous other nephrotoxic medications did not have a change in creatinine.

Kahata and colleagues did report a single patient with chronic myelogenous leukemia in June 1994 who had an adverse reaction to inhaled vancomycin while preparing to receive an allegeneic bone marrow transplant[16]. Thirty minutes after inhaling vancomycin (dose unknown) the patient developed cough, fever, shortness of breath, eosinophilia, hypoxia, and elevated CRP. The symptoms completely resolved with steroids.

There have also been studies of achievable sputum levels after nebulization of vancomycin. In non-CF patients, mean sputum vancomycin levels after nebulizing 125mg were 262.5 μg/g at 10 minutes and 50 μg/g at 6 hours - both of which are still 25 times above a therapeutic MIC[13]. In another report of a non-CF mechanically ventilated patient, sputum vancomycin levels were 2,352 μg/mL 1 hour after nebulizing 120mg of vancomycin[17]. The higher level in the latter study may be related to breath actuated nebulization (as opposed to continuous) and turning off the humidifier during nebulization. There have not been any CF specific studies to date of achievable sputum levels after inhaled vancomycin.

Finally, there is significant clinical experience with the use of nebulized vancomycin in CF, as the CF treatment community frequently uses nebulized vancomycin in clinical care. Foundation Care, a compounding pharmacy which supplies CF healthcare providers nebulized vancomycin for clinical care, has documented treatment of over 95 CF patients with the dose described in the protocol without any treatment related serious adverse events. Please see Appendix 2 for a table summarizing known safety and efficacy of inhaled vancomycin reported in over 180 patients.

2 STUDY RATIONALE

2.1 Importance of MRSA in Cystic Fibrosis

The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) respiratory infection in CF has increased dramatically over the last decade, with over 25% of CF patients now having pulmonary infection with MRSA[3]. Epidemiologic evidence suggests that persistent infection with MRSA is an independent risk factor for an increased rate of decline in FEV_1 and shortened survival[4;5].

2.2 Lack of Conclusive Clinical Trials of MRSA Therapy Protocol in CF

Currently there are no conclusive studies demonstrating an effective aggressive treatment protocol for persistent MRSA respiratory infection in CF. Pilot data demonstrating an effective and safe method of clearing MRSA infection are needed. While there have been several small clinical studies in CF assessing MRSA treatment regimens, these studies have been limited by small study populations, single-center design, retrospective design, variable follow-up, and failure to distinguish incident vs. persistent MRSA infection.

2.3 Rationale for Specific Drugs included in the Protocol

2.3.1 Why inhaled vancomycin?

1) Multiple reports in CF and outside of CF have suggested vancomycin to be an effective method of treating pulmonary MRSA infection.
2) Multiple reports in CF and outside of CF have suggested inhaled vancomycin to be a safe and well tolerated method of treating pulmonary MRSA infection while minimizing systemic absorption.

2.3.2 Why oral rifampin?

1) Rifampin has excellent mucosal penetration and has been shown to clear MRSA when added to vancomycin when vancomycin alone failed [18]
2) Rifampin in combination with vancomycin has been shown to be bactericidal against biofilm producing MRSA[19])
3) Rifampin in combination with another antibiotic eradicated MRSA in previous CF studies[8;11].

2.3.3 Why Mupirocin Nasal and 4% Chlorhexidine Gluconate Skin Treatment and Sani-Cloth Alcohol Free Germicidal Wipes?

1) CF patients are known to have a significantly higher rate of staph aureus anterior nasal carriage compared to non-CF controls (66% vs. 32%)[20].
2) Mupirocin nasal treatment has been demonstrated in non-CF studies to successfully eradicate nasal colonization of MRSA in 93% of patients[21].
3) Stone and colleagues recently published a cross sectional study which demonstrated that *S. aureus* from the anterior nares may be transmitted between CF patients and household contacts[22]. In their study, 15% of household contacts of MRSA positive CF patients cultured MRSA from their nares. Therefore we have elected to treat high touch home environment with chlorhexidine wipes and provide a handout with information on decreasing transmission within the home environment. Sani-Cloth Alcohol Free Germicidal Wipes are documented to disinfect surfaces from MRSA and are fragrance free to avoid respiratory irritation.
4) Recent review of regimens for the decolonization of MRSA concluded 4% chlorhexidine gluconate whole body washes in combination with mupirocin to be the regimen of choice[23].

3 STUDY OBJECTIVES

3.1 Primary Objectives

The primary objectives of this trial are to:

1) Determine the efficacy of an aggressive treatment protocol in eradicating persistent MRSA infection in individuals with CF.
2) Determine the safety of an aggressive treatment protocol in eradicating persistent MRSA infection in individuals with CF.

3.2 Secondary Objectives

The secondary objectives of this trial are to:

1) Determine the efficacy of an aggressive treatment protocol in improving FEV1, time to exacerbation, and quality of life in individuals with CF and persistent MRSA infection.

2) Determine if there is benefit to adding nebulized vancomycin to an aggressive oral antibiotic treatment protocol in eradicating persistent MRSA infection in individuals with CF.

4 STUDY DESIGN

4.1 Study Overview

This is a two-center, double-blind, comparator-controlled, randomized, and stratified on center and $FEV_1\%$ ($FEV_1\% \leq 60\%$ and $FEV_1\% > 60\%$) parallel-group study with 1:1 assignment to either vancomycin for inhalation (250mg twice a day) or taste matched placebo for 28-days in individuals with cystic fibrosis and chronic MRSA pulmonary infection. In addition, both groups will receive oral rifampin, a second oral antibiotic (TMP-SMX or doxycycline, protocol determined), mupirocin intranasal ointment and chlorhexidine body washes. 40 patients with persistent respiratory tract MRSA infection will be enrolled: 20 will be randomized to vancomycin for inhalation and 20 to a taste-matched placebo.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

Each subject will be administered study medication twice a day for 28 days. Subjects will be randomly assigned to the treatment or placebo. Evaluations will be taken at each of the 5 study visits.

All subjects who receive at least one dose of study medication will be considered evaluable for safety and efficacy analyses. Incidence of adverse events will be monitored beginning at the time of consent.

Efficacy assessments will be based on culture results, changes in lung function and sputum MRSA density in sputum, CFQ-R scores, and exacerbations.

Total duration of subject participation will be 146 ± 9 days. Total duration of the study is expected to be 48 months.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Percentage of patients MRSA free by induced sputum respiratory tract culture 1 month (Visit 5, Day 58) after completion of four-week eradication protocol in intervention arm vs. control arm.

5.2 Secondary Efficacy Endpoints

- Percentage of patients MRSA free at Visit 4 (Day 29) and Visit 6 (Day 118).
- Change in FEV_1 from baseline (Day 1) at Visit 4 (Day 29), Visit 5 (Day 58) and Visit 6 (Day 118).
- Change in FEV_1 from Screening (Day -28) at Visit 4 (Day 29), Visit 5 (Day 58) and Visit 6 (Day 118).
- Time to first exacerbation (standard definition in appendix 3).
- Time to first anti-MRSA antibiotics (after treatment period)
- Total number of exacerbations at Visit 5 (Day 58) and Visit 6 (Day 118).
- Change in patient reported CFQ-R (respiratory) from baseline (Visit 1) to Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (Day 58), and Visit 6 (Day 118).
- Number of patients with newly developed MRSA resistance to vancomycin, TMP/SMX, doxycycline, and/or rifampin at any point during follow-up.

5.3 Safety Evaluations

- Chemistry panel, AST, ALT, alkaline phosphatase, and CBC with differential, at Screening (Day -28), Visit 1 (Day 1), Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (Day 58) and Early Withdraw Visit.
- Incidence of adverse events.
- Continuous oxygen saturation throughout nebulized vancomycin dose at Visit 1 (Day 1), Visit 2 (Day 2-7) and Visit 3 (Day 14).
- Spirometry checked immediately after and 15 minutes (+/-10min) after nebulized vancomycin dose at Visit 1 (Day 1) and Visit 2 (Day 2-7).
- Hand washing with chlorhexidine body wash at Run In Visit (Day-14) to monitor for skin sensitivity.

5.4 Other Evaluations

- Serum vancomycin peak level 60 minutes after completion of first dose of inhaled vancomycin at Visit 1.
- Trough serum vancomycin level to be drawn prior to nebulization of vancomycin at Visit 3 (Day 14-two weeks into nebulized vancomycin).
- Sputum vancomycin level at Visit 1 (Day 1), 5±4 minutes after nebulizing vancomycin in patients.
- Change in minimum inhibitory concentration (MIC) of vancomycin, rifampin, TMP/SMX, and tetracycline for MRSA. As per CDC guidelines. VISA if the MIC for vancomycin is 4-8µg/ml, and classified as VRSA if the vancomycin MIC is ≥16µg/ml. We will also monitor for those MIC's that are initially less than 2 µg/mL and convert to 2-4 µg/mL.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of CF who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Male or female ≥ 12 years of age.
2. Confirmed diagnosis of CF based on the following criteria:
   - positive sweat chloride > 60 mEq/liter (by pilocarpine iontophoresis) and/or
   - a genotype with two identifiable mutations consistent with CF or abnormal NPD, and
   - one or more clinical features consistent with the CF phenotype.
3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.
4. Two positive MRSA respiratory cultures in the last two years at least six months apart, plus a positive MRSA respiratory culture at Screening Visit and Run-in (Day -14) Visit.
5. At least 50% of respiratory cultures from the time of the first MRSA culture (in the last two years) have been positive for MRSA.
6. FEV1 ≥40% of predicted normal for age, gender, and height at Screening, for subjects 18 years of age and older.
7. FEV1 ≥60% of predicted normal for age, gender, and height at Screening, for subjects 12-17 years old.
8. Females of childbearing potential must agree to practice one highly effective method of birth control, including abstinence. Note: highly effective methods of birth control are those, alone or in

combination, that result in a failure rate less than 1% per year when used consistently and correctly. Female patients who utilize hormonal contraceptives as a birth control method must have used the same method for at least 3 months before study dosing. If the patient is using a hormonal form of contraception and is receiving rifampin, patients will be required to also use barrier contraceptives as rifampin can affect the reliability of hormone therapy. Barrier contraceptives such as male condom or diaphragm are acceptable if used in combination with spermicides.

6.3 Exclusion Criteria

1. An acute upper or lower respiratory infection, pulmonary exacerbation, or change in routine therapy (including antibiotics) for pulmonary disease within 42 days of the Day 1 Visit (2 weeks prior to Screening visit).
2. Individuals on chronic continuous inhaled antibiotics without interruption who are not willing to substitute vancomycin or placebo for their scheduled inhaled antibiotic during days 0-28 of the study (every other month inhaled antibiotics are acceptable)
3. Use of oral or inhaled anti-MRSA drugs within two weeks of the Screening Visit.
4. History of intolerance to inhaled vancomycin or inhaled albuterol.
5. History of intolerance to both TMP/SMX and doxycycline.
6. Resistance to both TMP/SMX and doxycycline at Screening.
7. Resistance to vancomycin at Screening.
8. Abnormal renal function, defined as creatinine clearance <50 mL/min using the Cockcroft-Gault equation for adults or Schwartz equation in children, at Screening.
9. Abnormal liver function, defined as ≥3x upper limit of normal (ULN), of serum aspartate transaminase (AST) or serum alanine transaminase (ALT), or known cirrhosis. at the time of Screening.
10. Serum hematology or chemistry results which in the judgment of the investigator would interfere with completion of the study.
11. History of or listed for solid organ or hematological transplantation
12. History of sputum culture with non-tuberculous *Mycobacteria* in the last 6 months.
13. History of sputum culture with *Burkholderia Cepacia* in the last year.
14. Planned continuous use of soft contact lenses while taking rifampin and no access to glasses.
15. Current use of oral corticosteroids in doses exceeding the equivalent of 10 mg prednisone a day or 20 mg prednisone every other day
16. Administration of any investigational drug or device within 28 days of screening or within 6 half-lives of the investigational drug (whichever is longer)
17. Patients on inhaled antibiotics who have not been on the same regimen for the 4 months prior to screening.
18. Female patients of childbearing potential who are pregnant or lactating, or plan on becoming pregnant
19. Any serious or active medical or psychiatric illness, which in the opinion of the investigator, would interfere with patient treatment, assessment, or adherence to the protocol.

7 CONCURRENT MEDICATIONS

All subjects should be maintained on a stable medical regimen throughout the entire study period, as medically feasible, with the exception that patients on continuous inhaled antibiotics will stop their non-study drug inhaled

antibiotic during the 28 day treatment period. If subjects are on an alternating month inhaled antibiotic, study drug dosing will occur during an "off" cycle. There should be no introduction of other new chronic therapies during the study period unless medically required.

7.1 Allowed

All usual CF medications and treatments are allowed except anti-MRSA antibiotics beginning two weeks prior to the screening visit and throughout the study (unless medically indicated).

7.2 Prohibited

Inhaled antibiotics other than study drug from Day 1 through Day 28

Unless medically indicated, intravenous antibiotics from Day -42 through Day 28.

Regular use of loop diuretics during days -14 through 28.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Up to 40 eligible patients will be randomly assigned to vancomycin for inhalation or placebo treatment (Days 1-28) in a 1:1 ratio using a SAS-based computer-generated randomization scheme developed by a biostatistician at Case. The randomization will be performed in blocks of random sizes and stratified by FEV_1% and center. The randomization will also be designed to assure an equal division between placebo and treatment among the first sixteen participants in preparation for the first DSMB safety review (after 16 participants are enrolled). The investigator or designee will complete a randomization worksheet (a Non-visit task at Day -4).

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments.

Access to the randomization code will be strictly controlled. Access to the randomization code will be limited to the randomization programmer, Manager of the Data Management Unit as a backup to the randomization programmer, and the drug packaging group.

Packaging and labeling of test and control treatments will be identical.

Since vancomycin levels in the serum and sputum will be measured, the results of these tests will not be available to the investigators.

The study blind will be broken on completion of the clinical study and after all adverse events have been evaluated for relationship to study drug, coded, and reviewed by the DSMB.

During the study, the blind may be broken **only** in emergencies when knowledge of the patient's treatment group is necessary for further patient management. When possible, the Investigator should discuss the emergency with the DSMB prior to unbinding.

8.3 Formulation of Study Medication (Vancomycin/Placebo)

Study Medication: Vancomycin hydrochloride is a tricyclic glycopeptide antibiotic obtained from *Amycolatopsis orientalis*. It is an off-white lyophilized powder that is oxygen sensitive. The manufacturer is Hospira, Inc. (Lake Forest, IL).

Classification: Antibiotic

Route of Administration: The compounded sterile preparation is intended for inhalation via a nebulizer device.

Method of preparation: The dispensing pharmacy will be Foundation Care (Earth City, MO). Foundation Care is an FDA inspected facility and is also accredited by the Pharmacy Compounding Accreditation Board. All preparations of vancomycin for inhalation are done in a sterile compounding room based on USP policies and procedures via aseptic technique. Vancomycin will be reconstituted by adding 20 mL of sterile water to a 1g vial of sterile vancomycin powder. Each batch of sterile vancomycin (1g/vial) will have a certificate of analysis assuring the identity, strength, quality, and purity of the drug substance [see Appendix 4 Figure 1 (Example Hospira certificate of analysis)] and be packaged in tamper proof, individual, single-dose (250 mg) plastic ampules. [See Appendix 4 Figure 2 (Example of compounding formula record) and Appendix 4 Figure 3 (Description of the manufacturing and packaging procedures)].

The final product will undergo a series of analytical assays to determine the identity, strength, quality, and purity. Before the vancomycin for inhalation can leave the facility, a series of checks and tests in accordance with Standard Operating Procedures after compounding is performed to ensure sterility and accuracy. Each ampule is visually inspected for integrity, appropriate solution color, volume, particulates, cloudiness, and container leakage before leaving the clean room. The product is double checked before dispensing. A sample vial from each batch is sent to test for sterility, fungus, yeast, molds, and endotoxins. A preliminary report is returned after 72 hours [see Appendix 4 Figure 4 (Example microbiology report) and Appendix 4 Figure 5 (Example certificate of analysis)].

Stability and Storage: Stability testing has been previously performed. The beyond use period has been set at 60 days based upon prior testing of potency at 0, 30, 60, and 90 days after preparation. Vancomycin for inhalation should be stored in a refrigerator at a temperature of 2-8°C. Based on prior validation, the product is potent and stable for greater than 60 days when stored between 2-8°C.

Study Placebo: The placebo will be 5cc of sterile water and 0.5 mg of quinine for taste masking to maintain blind.

Route of Administration: The compounded sterile preparation is intended for inhalation via a nebulizer device.

Method of Preparation: The placebo will also be prepared by Foundation Care in sterile fashion as described above with identical analytical assays to determine quality and purity. Each ampule is visually inspected for integrity, appropriate solution color, volume, particulates, cloudiness, and container leakage before leaving the clean room. The product is double checked before dispensing. A sample vial from each batch is sent to test for sterility, fungus, yeast, molds, and endotoxins. A preliminary report is returned after 72 hours.

The placebo formulation is based on previous recommendations by the FDA on inhaled placebo design to the investigators of the Cystic Fibrosis Foundation Inhaled Hypertonic Saline Trial. (N.Engl.J.Med. 354, 241-50, 2006, personal communication with Donaldson). We will be using less quinine for taste masking of the sterile water than was used in that trial. (0.1 mg of sterile quinine per ml of sterile water in this trial vs. 0.25 mg of sterile quinine per ml of sterile water in the Hypertonic Saline Trial.) Similar to that study, the quinine used will be sterile, USP grade, manufacturer DSM Minera.

Storage: Identical to the vancomycin study drug, the placebo inhalation will be stored in a refrigerator at a temperature of 2-8°C during use.

8.3.1 Packaging and Labeling

Study drug is supplied in cartons containing 60 single use ampules. Any excess study drug will be collected at Visit 4 (Day 29), stored by the investigative pharmacy, and destroyed at the end of the study or during an interim monitoring visit.

Labeling: Each carton (kit) of study drug will be labeled with drug name (vancomycin/placebo), a lot number, an expiration date, the protocol name, directions for storage, and the words “For investigational use only”.

8.3.2 Handling/Dispensing

Study drug should be stored securely under refrigeration both in the investigational pharmacy and at the patient’s home. The investigator and/or the pharmacist have the authority to dispense drug, and each site’s

investigational pharmacy will oversee the dispensing and study drug/placebo supply. Drug should be dispensed by the research pharmacy within 28 days of shipment from the central pharmacy.

8.3.3 Dosage/Dosage Regimen

Nebulized Vancomycin: 250 mg nebulized two times a day for 28 days in 5cc sterile water. The selection of this dose is based on previous experience in both studies in cystic fibrosis and clinical experience by Foundation Care (see appendix 2). Patients will use a Pari LC Sprint Nebulizer cup and Pari Vios Compressor delivery system. Doses should be taken at least 4 hours apart. After the first 16 adult patients have been enrolled, the DSMB will review the safety data and if indicated will allow the inclusion of individuals with CF aged 12-17, as MRSA infection is a significant pathogen in this group. Based on the studies by Solis[10] and Generali[12], and previous clinical experience by Foundation Care, the dosing for this group will also be 250 mg nebulized two times a day for 28 days in 5cc sterile water. This is consistent with current FDA Approved nebulized antibiotic use in Cystic Fibrosis patients with inhaled tobramycin (TOBI) and Aztreonam (CAYSTON), in which identical doses are used in adult and pediatric populations given the minimal systemic absorption.

8.3.4 Administration Instructions Nebulized Vancomycin/Placebo (Days 1-28)

Patients who meet the inclusion criteria and are randomized at non-visit Day -4 will be dispensed a 28 day supply of Vancomycin 250mg twice a day or matched placebo on Visit 1 (Day 1). Patients will be provided with two Pari LC Sprint nebulizer cups and a Pari Vios compressor which should be used to administer study or control medication. In addition, patients will be provided with a Pari Expiratory Filter/Valve Set with 60 single use expiratory filter pads to be used with each drug administration. Patients will be provided with an albuterol HFA and instructed to take two puffs at home 5 to 30 minutes prior to study drug inhalation. The treatment period is 28 days. Witnessed study drug administration will occur in the clinic at Visit 1 (Day 1). Repeat witnessed administration will occur in the clinic at Visit 2 (Day 2-7) and Visit 3 (Day 14). The witnessed dose will be placed into the opaque nebulizer cup by the subject to prevent the RC or physician from examining the characteristics of the study drug. Subsequent doses will be self-administered twice a day (morning and evening). If a dose is missed, both doses should be taken as long as they are at least 4 hours apart. Order of inhaled medications/treatments when at home:

1) Albuterol HFA (if patients already use albuterol nebulizers or levalbuterol, those medications can be substituted here)
2) Hypertonic saline (if applicable)
3) Dornase alfa (if applicable)
4) Chest physiotherapy (VEST, flutter, acapella, or other airway clearance method, if applicable)
5) Study or control medication
6) Inhaled corticosteroids (if applicable)

Patients will be instructed to refrain from using short acting bronchodilators 4 hours prior to appointment. All patients will receive a short acting bronchodilator prior to spirometry and study drug dosing at study Visit 1, 2 and 3. On other visit days, participants will receive bronchodilator prior to spirometry and not have further bronchodilator prior to induced sputum unless it occurs more than 2 hours after previous bronchodilator use.

Along with their nebulizers, patients will also be provided with nebulizer cleaning instructions.

8.4 Supply of Study Medication at the Site

Once the results of the sputum culture from Run-In Visit 2 (Day -14) is confirmed positive, the participant will be randomized by the Foundation Care pharmacy utilizing a predesigned randomization sheet and the Foundation Care pharmacy will dispense study or control drug kits to the site Investigational Drug Service Pharmacy. Study medication will be sent overnight packed in dry ice labeled for investigational use only.

8.4.1 Storage

Study drug should be stored securely under refrigeration both in the investigational pharmacy and at the patient's home. The investigator and/or the pharmacist have the authority to dispense drug. Drug should be dispensed within 28 days of shipment from the central pharmacy to IDS.

8.5 Study Medication Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the Investigational Drug Service Pharmacy at each site. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Subjects will be asked to keep a patient diary noting if study drug was taken as directed, refrigeration of the study drug, any adverse events. They will be asked to bring their patient diary to each study visit along with all used and unused study drug containers.

8.7 Other Protocol Prescribed Treatments:

8.7.1 **Oral Rifampin** (Lannett Company, Philadelphia PA) **(Days 1-28).** Participants will be treated with oral rifampin on days 1-28 according to the following dosing guidelines:

>45 kg:	Rifampin 600 mg by mouth daily
35-45 kg:	Rifampin 450mg by mouth daily
25-34.9 kg:	Rifampin 300mg by mouth daily

Participants with known allergies, and/or intolerances to rifampin will forgo treatment with this antibiotic, at the discretion of the investigator. Additionally, any participant whose current CF treatment regimen includes medication(s) that are known to produce adverse drug-drug interactions with rifampin will forgo treatment with rifampin. Participants with evidence of MRSA resistance to rifampin at Screening will not be treated with this antibiotic. Rifampin will be dispensed as a combination of 300 mg and 150 mg capsules to create the protocol determined dose. Rifampin may be stored at room temperature. Rifampin is known to potentially cause gastroesophageal reflux symptoms (GERD). For individuals experiencing this, an adverse event will be recorded and treatment will instituted by either increasing current GERD therapy or instituting treatment with a proton pump inhibitor. The proton pump inhibitor will be ordered by the patient's primary CF physician. Continued symptoms judged by the investigator or participant as more than mild despite treatment will result in discontinuation of the rifampin, although participants will be permitted to remain in the study.

8.7.2 **Protocol Determined Oral Antibiotic - TMP/SMX (Mutual Pharmaceutical Company, Philadelphia, PA) or Doxycycline** (Keltman Pharmaceuticals, Flowood, MS) **(Days 1-28)** . All participants will be treated with either TMP/SMX or doxycycline on days 1-28 according to the following protocol:

Participants with MRSA susceptible by MIC testing to TMP/SMX and without history of sulfa allergy will be treated with trimethoprim/sulfamethoxazole (DS-160/800):

>45 kg:	Two DS tablets twice a day by mouth (320/1600)
25-45 kg:	One DS tablet twice a day by mouth (160/800)

Participants with MRSA resistant by MIC testing to TMP/SMX and without history of doxycycline allergy will be treated with doxycycline:

>45 kg:	100 mg by mouth twice a day
35-45 kg:	75 mg by mouth twice a day
25-34.9 kg:	50 mg by mouth twice a day

Participants with evidence of only SCV MRSA at Screening will be treated with doxycycline, per the above dosing guidelines. For participants with both SCV MRSA and normal colony MRSA at Screening, antibiotic choice will be based on the following:

- SCV MRSA + normal colony MRSA susceptible to TMP/SMX and doxycycline: treat with doxycycline
- SCV MRSA + normal colony MRSA resistant to TMP/SMX and susceptible to doxycycline: treat with doxycycline
- SCV MRSA + normal colony MRSA susceptible to TMP/SMX and resistant to doxycycline: treat with TMP/SMX

8.7.3 **Mupirocin 2% Intranasal Ointment** (GlaxoSmithKline, Research Triangle Park, NC) **(Days 1-5).** All participants without known Mupirocin allergy will be treated with Mupirocin 2% intranasal ointment on days 1-5. Participants will use a total of 10 single use tubes of Mupirocin 2% intranasal tubes over 5 days by administering one half of a tube into each nasal cavity and pinching their nose closed for one minute. They will do this each morning and each evening for five days. Mupirocin allergy will not be an exclusion criterion.

8.7.4 **Hibiclens (4% chlorhexidine gluconate) Liquid Skin Cleanser** (Molnlycke Health Care, Norcross, GA) **(once a week for weeks 1-4).** All participants without known chlorhexidine gluconate allergy will be provided with 12 packets of 4% chlorhexidine gluconate for use of three packets once weekly in the shower during days 1-28. Participants will scrub with Hibiclens on a washcloth from the neck to the toes, with attention to the axilla, groin, and buttocks. Participants should not allow contact with skin above the neck. Individuals with a chlorhexidine allergy will not do the Hibiclens scrubs but this will not be an exclusion criterion to participation in the trial.

8.7.5 **Wipe down of high touch household surfaces with** Sani-Cloth Alcohol Free **Germicidal wipes** (PDI Incorporated, Orangeburg, NY) **(once a week for weeks 1-4).** All participants will be provided with a packet of Sani-Cloth Alcohol Free wipes and a box of gloves to wipe down high touch surfaces in their home. High touch surfaces include door handles, light switches, computer key boards, television sets/remote controls, telephones, kitchen and bathroom sink and counter areas, toilets/toilet seats. Performance of cleaning will be captured in the patient diary.

8.7.6 **Wash all linens and towels in hot water weekly for the first three weeks.** All participants will be instructed for days 1-21 of the study to wash weekly in hot water all bedding linens and towels with which they will have contact. Performance of linen washing will be captured in the patient diary.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject *or subject's legal representative*. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening, the Run-in Visit, Visit 1, Phone Visits, Visit 2, Visit 3, Visit 4, Visit 5, and Visit 6 and at Early Withdrawal Visit when applicable. Dose, route, unit, frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, medications, and information regarding underlying diseases will be recorded at Screening and all subsequent visits. In addition, at the screening visit we will determine history of previous treatment for MRSA, number of MRSA cultures, and length of MRSA positivity.

9.1.4 Physical Examination

A complete physical examination will be performed by a physician (either the principle investigator or a physician subinvestigator) at Screening , Visit 4, Visit 6, and the Early Withdrawal Visit (if applicable). The abbreviated physical exam may be completed by qualified staff (MD, NP, RN, PA) at all other visits. An abbreviated physical exam will consist of an oropharyngeal, skin, lung, cardiac, and abdominal exam. New abnormal physical exam findings will be documented and followed by a physician or other qualified staff.

9.1.5 Vital Signs and Height and Weight

Body temperature, blood pressure, pulse and respirations will be performed after resting for five minutes at all visits. Height and weight will be recorded at all visits.

9.1.6 Oximetry

Oximetry will be measured on room air with the subject at rest at all visits. During the observed nebulization of study drug Visits 1, 2 and 3, participant will undergo continuous oxygen saturation monitoring throughout the nebulization period and for 5 minutes afterward.

9.1.7 Spirometry

Spirometry will be performed at all visits in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests. Subjects will be given two inhalations of Albuterol HFA 10-30 minutes prior to initial spirometry. For the repeat spirometry immediately after and 15 minutes (± 10 mins) after dosing of study drug at Visit 1 and Visit 2, a single value will be acceptable if FEV_1 percent predicted improves, is stable, or has a relative decrease less than 10% from consistent baseline prior to nebulization.

9.1.8 CFQ-R

The CFQ-R (Cystic Fibrosis Quality of Life Questionnaire –Revised Assessment) is a validated CF-specific instrument that measures the health-related quality of life for patients with CF. Furthermore, the CFQ-R has been evaluated in clinical studies involving therapies for CF lung disease. The CFQ-R will be completed prior to any other interventions at Visits 1, 3, 4, 5 and 6 and Early Withdrawal Visit as applicable. A minimum clinically important difference of 4 points will be considered clinically meaningful for the respiratory domain[24].

9.1.9 Other Clinical Procedures

When required, sputum induction will be performed according to TDN SOP 530.02 or 535.01 (per nebulizer type) and Processing TDN SOP 508.02.

Subject Diary: Participants will complete the subject diary daily beginning at the Run in Visit (day -14), continue throughout the drug administration period and up until 14 days after completion of drug administration (Day 42). For diary details see Appendix 5.

Hibiclens hand wash: At the Day -14 Visit Participants will be provided one packet of Hibiclens hand cleanser and wash their hands with water and Hibiclens for 2 minutes. Skin reaction will be observed for 15 minutes after washing; subsequent development of skin irritation will be noted as an adverse event. Skin irritation will be reassessed as part of the Visit 1 physical exam. Individuals with evidence of intolerance of the Hibiclens wash will not do the whole body washes, but this will not be a reason for exclusion from the trial.

9.1.10 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study starting at the time of consent and ending at the last study visit. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study medication will be recorded on the case report form (CRF). Adverse events will be followed until resolution.

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained and sent to each site's local clinical hematology lab for a complete blood count with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count) at Screening, Visit 1, Visit 3, Visit 4, Visit 5 and Early Withdrawal Visit as applicable.

9.2.2 Blood Chemistry Profile and C-Reactive Protein

Blood will be obtained and sent to each site's local clinical chemistry lab for determination of serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), and albumin at Screening, Visit 1, Visit 3, Visit 4, Visit 5, and Early Withdrawal. In addition a C-Reactive Protein (CRP) will be collected at Screening, Visit 1, Visit 3, Visit 4, Visit 5, and Early Withdrawal Visit as applicable.

9.2.3 Pregnancy Test

A serum pregnancy test will be obtained from female subjects who are of childbearing potential at the Screening Visit. A urine pregnancy test will be performed prior to nebulization of study drug at study Visits 1 and 3.

9.3 Pharmacokinetic Measurements

Blood for determination of serum concentrations of vancomycin will be collected 60 minutes (±10 mins) after the start of dosing at Visit 1 (Day 1) and a trough level prior to administration of nebulized vancomycin will be obtained on Visit 3. Vancomycin serum concentrations will be determined at each site's clinical chemistry lab using a standard ELISA method. Investigators will be blinded to the results, however the laboratory will be instructed to contact the investigator and report any trough value in excess of 8 µg/ml. Results will be collected by the laboratory and forwarded to the DSMB for review.

9.4 Research Laboratory Measurements

9.4.1 Cell Count and Differential

9.4.2 Sputum Cell Counts and Differential and Cytokine Measurements

Sputum for determination of neuregulin-1, IL-8, IL-1B, and elastase will be collected at Run-in (Day -14) and Visit 4 (Day 29). Sputum for determination of cell count will be collected at Run-in (Day -14), Visit 3 (Day 14), Visit 4 (Day 29), and Visit 5 (Day 58). Specimens will be collected in a sterile specimen cup. The cup will be labeled, packed in ice and shipped overnight to the Core Laboratory of the Cystic Fibrosis Foundation at Rainbow Babies and Children's Hospital for processing

9.4.3 Quantitative Bacteriology

Sputum will be collected for culture and sensitivity at Screening, Run-in (Day -14), Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (Day 58), and Visit 6 (Day 118) and Early Withdrawal visit if necessary. All sputum specimens should be collected in a sterile specimen cup, labeled with patient study number and initials, and sent to the study's microbiology core laboratory in Seattle for culture including identification of MRSA small colony variants. Specimens will be processed within 2 days of collection. Quantitative culture for MRSA colony forming units (CFU's) will be performed on each microbiologic specimen at Screening, Run-in (Day -14), Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (Day 58) and Visit 6 (Day 118). CFU's will be obtained at the Early Withdrawal Visit if the visit occurs between Visit 3 and Visit 5. Susceptibility testing for rifampin, vancomycin, tetracycline, and TMP/SMX will be performed on all MRSA isolated from samples collected. MRSA comprehensive genetic strain analysis will be performed at Screening, Run-in (Day -14), Visit 3 (Day 14), Visit 4 (Day 29), Visit 5 (Day 58), and Visit 6 (Day 118) and Early Withdrawal Visit if necessary. If induced sputum fails to produce sputum, a throat culture will be obtained and evaluated for the presence of MRSA.

9.4.4 Nasal and Axillary Cultures

At the Run-in Visit (Day -14), Visit 4 (Day 29), and Visit 5 (Day 58) separate nasal and axillary cultures will be taken by rotating one culture swab in both nostrils and a separate culture swab in the left axilla. These will be assessed for the presence of MRSA in each site's local microbiologic laboratory.

9.4.5 Rectal Culture (Optional)

At the Run-in Visit (Day -14) and at Visit 4 (Day 29) an optional rectal culture will be obtained in individuals age 18 or older who agree to participate by having the patient rotate one culture swab in the anal opening. These will be assessed for the presence of MRSA and Vancomycin resistant enterococcus in each site's local microbiologic laboratory.

9.4.6 Sputum Vancomycin Measurements

Spontaneous expectorated sputum will be collected for vancomycin peak measurements at Visit 1 (Day 1). After completion of nebulization of study drug, patient will immediately gargle three times with 30 mL normal saline solution to avoid contamination of specimens by drug deposited in the oropharynx. All sputum specimens should be collected in a sterile specimen cup and labeled with patient study number and initials. Specimens from Case will be placed stored in a -80°C freezer in the research center and then walked over to the Case Western Reserve University Research Pharmacology Lab. Specimens from Hopkins will be stored in a -80°C freezer until shipped overnight on ice to the Case Western Reserve University Research Pharmacology Lab.

9.5 Guidelines for Timing of Use of Chronic Oral and Inhaled Antibiotics

Patients on alternating month chronic inhaled antibiotics (same regimen for at least 4 months) (i.e. TOBI, Cayston, Colistin) will be invited to the Screening Visit during the beginning of their "on" month. All patients will go through the 28 day screening period while on their chronic inhaled antibiotic therapy. Study drug dosing will occur during an "off" cycle. For those patients on chronic (same regimen for at least 4 months) continuous

inhaled antibiotics, they would have to be willing to hold their inhaled antibiotics during the study period. To minimize differences between groups, patients would be required to screen during their TOBI month. If a patient is not on TOBI, but is on two other inhaled antibiotics, then they would have to screen while on colistin. If a patient is not on either TOBI or colistin, then patients would have to screen on Cayston.

Individuals may not have been on any oral anti-MRSA antibiotics in the two weeks prior to the Screening Visit. These include any of the oral antibiotics in the protocol (rifampin, TMP/SMX, doxycycline) and clindamycin, minocycline, tetracycline, ciprofloxacin, moxifloxacin, or any other antibiotic with known anti-MRSA effect. After the 28-day treatment protocol ends, participants may not be started on oral anti-MRSA antibiotics unless indicated by symptoms and MRSA has been documented to be present in their sputum by culture.

9.6 Guidelines for Treatment of CF Exacerbations During the Study

Participants that experience a pulmonary exacerbation during the 28 day study drug treatment period will be assessed by another clinical caregiver on the CF team besides the study PI. If in the assessment of that caregiver the participant is in need of addition of other antibiotics for best clinical care, the participant will be discontinued from the study and treated. They will be replaced in the study, but their data will be utilized in the calculation of the exacerbation outcomes if they received more than 14 days of nebulized study drug.

Participants that experience a pulmonary exacerbation which occur after completion of the 28 day study drug period will also be assessed by another clinical caregiver on the CF team besides the study PI. If in the assessment of that caregiver the participant is in need of addition of other antibiotics for best clinical care, the participant will be treated with IV or oral antibiotics determined by their most recent available respiratory culture. They will remain in the study and their data will be utilized in the calculation of eradication and exacerbation outcomes.

10 EVALUATIONS BY VISIT

10.1 Screening Visit (Day -28) (±2 days)

1. Before any study procedures are performed, review the study with the subject and/or subject's legal representative and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including a history of CF, diagnosis date, and prior CF treatments including treatment for MRSA. Record the dates of all previous MRSA cultures for the previous three years and date of first positive MRSA culture ever (if available).
5. Review inclusion and exclusion criteria to assure patient eligible to participate
6. Record concomitant medications with particular attention to potential drugs which may interact with rifampin, doxycycline or TMP/SMX.
7. Perform and record vital signs, height and weight.
8. Perform and record oximetry.
9. Perform a complete physical examination.
10. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
11. Perform and record spirometry (make sure meets inclusion criteria before performing blood tests).
12. Collect blood for clinical laboratory tests (chemistry, hematology, LFT's, CRP, pregnancy test, if applicable).
13. Induce and collect sputum into one cup for bacterial culture, antibiotic sensitivity, MRSA colony forming units, and small colony variants of MRSA, and comprehensive genetic strain analysis.
14. Schedule subject for Run-in Visit in 14 days (± 4 days).

10.2 **Run-In Visit (Day -14) (± 4 days)**

1. Record follow-up medical history.
2. Record any adverse event
3. Concomitant medications review.
4. Perform and record vital signs, height and weight.
5. Perform and record oximetry
6. Perform abbreviated physical examination.
7. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
8. Perform and record spirometry.
9. Obtain axillary and nasal swab for MRSA (to be submitted to local microbiology lab).
10. Obtain optional rectal swab for presence of Vancomycin Resistant Enterococcus.
11. Induce and collect sputum into one cup for bacterial culture, antibiotic sensitivity, MRSA colony forming units, and small colony variants of MRSA, and comprehensive genetic strain analysis and one cup for cell count and cytokine measurements.
12. Test hand washing with Hibiclens cleaner for two minutes and review skin response fifteen minutes later.
13. Initiate subject diary
14. Schedule subject for Visit 1 in 14 days (± 4 days).

10.2b **Non-visit task at Day -4 (±3 days):** Review Culture Results from Run-in Visit, confirm presence of MRSA, perform randomization.

10.3 **Visit 1 Day 1 (Start Treatment)**

1. Perform CFQ-R.
2. Record follow-up medical history.
3. Record any adverse events
4. Concomitant medications review.
5. Review subject diary
6. Perform abbreviated physical examination.
7. Perform and record vital signs including height and weight.
8. Perform and record oximetry.
9. Perform urine pregnancy test if applicable. If positive, patient is withdrawn from the study.
10. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry
11. Perform and record spirometry.
12. Initiate continuous oxygen saturation monitoring to be continued throughout study drug nebulization and for five minutes afterward.

13. Nebulize first dose of study drug utilizing expiratory neb cup with expiratory filter in place until sputtering or 15 minutes, whichever comes first.
14. After completion of nebulization, have participant immediately gargle three times with 30 mL normal saline solution for 10 s and expectorate the solution to avoid contamination of subsequent specimens by drug deposited in the oropharynx.
15. Immediately after gargling, perform and record spirometry (single value acceptable if within FEV_1 percent predicted improves, is stable, or has a relative decrease less than 10% from baseline).
16. Immediately after performing spirometry, obtain expectorated sputum vancomycin level and record time of collection. Goal is to collect sputum within 5 minutes (± 4 minutes) after nebulization of study drug. If patient unable to expectorate sputum, then no sputum collected for this measurement.
17. Perform and record spirometry 15 ± 10 minutes after completion of study drug (single value acceptable if FEV_1 percent predicted improves, is stable, or has a relative decrease less than 10% from baseline). Patient to be observed at least one hour post completion of nebulization.
18. Collect blood to obtain serum vancomycin level 60 (± 10) minutes after completion of nebulized study drug and collect blood for clinical laboratory tests (chemistry, hematology, LFT's, CRP).
19. Record serial number of compressor on source document.
20. Dispense PARI Vios compressor and LC Sprint neb cups (2) and Expiratory Filter and nebulizer cleaning instructions.
21. Dispense nebulized study drug and provide teaching on nebulization of study drug
22. Dispense protocol determined antibiotics, nasal mupirocin, and Hibiclens.
23. Provide teaching about protocol determined antibiotics.
24. Have participant take protocol determined oral antibiotics; provide snack as needed.
25. Provide teaching about nasal mupirocin.
26. Have participant use nasal mupirocin.
27. Provide teaching about Hibiclens wash.
28. Provide Sani-Cloth Alcohol Free Germicidal wipes, gloves and teaching about wiping high touch surfaces and washing towels and bed linens.
29. Schedule subject for Visit 2 in 2-7 days.

10.4 Visit 2 Day 2-7 (Repeat Witnessed Administration of Study Drug)

1. Record follow-up medical history.
2. Record any adverse events
3. Concomitant medications review.
4. Review subject diary
5. Perform and record vital signs including height and weight.
6. Perform abbreviated physical examination.
7. Perform and record oximetry.
8. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry

9. Perform and record spirometry.
10. Initiate continuous oxygen saturation monitoring to be continued throughout study drug nebulization and for five minutes afterward.
11. Nebulize first dose of study drug until sputtering or 15 minutes, whichever comes first.
12. Within five minutes of completion of nebulization, perform and record spirometry (single value acceptable if FEV_1 percent predicted improves, is stable, or has a relative decrease less than 10% from baseline).
13. Perform and record spirometry 15 ± 10 minutes after completion of study drug (single value acceptable if FEV_1 percent predicted improves, is stable, or has a relative decrease less than 10% from baseline).
14. Observe participant for at least one hour after administration of study drug.
15. Schedule phone call to occur on Day 7-14
16. Schedule subject for Visit 3 at day 14 (± 2 days)

10.5 Day 7-14 Phone Call or Email

1. Record any adverse events
2. Subject Diary Review
3. Concomitant Medication Review

10.6 Visit 3 Day 14 (± 2 days) (Mid-point Treatment)

1. Perform CFQ-R.
2. Record follow-up medical history
3. Record any adverse events.
4. Record changes to concomitant medications.
5. Collect all used study medication and containers; return all unused study medication and containers to subject for continued self-administration.
6. Review subject diary for adverse events and dosing adherence.
7. Perform and record vital signs, height and weight.
8. Perform and record oximetry.
9. Perform abbreviated physical examination.
10. Perform urine pregnancy test if applicable. If positive, patient is withdrawn from the study.
11. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
12. Perform and record spirometry.
13. Induce and collect sputum in one cup for bacterial culture, antibiotic sensitivity, MRSA colony forming units, small colony variants of MRSA and MRSA comprehensive genetic strain analysis, and another cup for cell count
14. Collect blood sample for clinical laboratory tests and serum vancomycin level and record time of collection: serum vancomycin level, chemistry, LFTs, hematology, and CRP.

15. Administer nebulized study drug until sputtering or 15 minutes, whichever comes first.
16. Have participant take protocol determined oral antibiotics (if they haven't already taken today); provide snack as needed.
17. Schedule phone call visit 2 and 3 to occur on Day 14-21 and Day 21-28
18. Schedule subject for Visit 4 on Day 29 (± 1 day)

10.7 Day 14-21 Phone Call or Email

1. Record any adverse events.
2. Subject Diary Review
3. Concomitant Medication Review

10.8 Day 21-28 Phone Call or Email

1. Record any adverse events.
2. Subject Diary Review
3. Concomitant Medication Review

10.9 Visit 4 Day 29 (± 1 day) (End treatment)

1. Perform CFQ-R.
2. Record follow-up medical history
3. Record any adverse events.
4. Record changes to concomitant medications.
5. Review subject diary for adverse events and dosing adherence. Provide subject with dairies to be continued for 14 days after end of treatment (through Day 42.)
6. Perform complete physical examination including height and weight.
7. Perform and record vital signs.
8. Perform and record oximetry.
9. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
10. Perform and record spirometry.
11. Collect blood sample for clinical laboratory tests: Chemistry, LFTs, Hematology, and CRP.
12. Obtain axillary and nasal swab for MRSA.
13. Obtain optional rectal swab for presence of Vancomycin Resistant Enterococcus.
14. Induce and collect sputum in one cup for bacterial culture, antibiotic sensitivity, MRSA colony forming units, small colony variants of MRSA, MRSA comprehensive genetic strain analysis and another cup for sputum cell count and cytokine measurements,
15. Collect all used and unused study medication and containers and Subject Diary.
16. Schedule subject for Visit 5 on Day 58 (± 4 days)

10.10 Visit 5 Day 58 (± 4 days) - (1 Month Post-treatment Monitoring Visit) (Primary Outcome)

1. Perform CFQ-R.
2. Record follow-up medical history
3. Record any adverse events.
4. Record changes to concomitant medications.
5. Review subject diary through Day 42 for adverse events.
6. Perform and record vital signs, height and weight.
7. Perform and record oximetry.
8. Perform abbreviated physical examination.
9. Collect blood sample for clinical laboratory tests: Chemistry, LFTs, Hematology, and CRP.
10. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
11. Perform and record spirometry.
12. Obtain axillary and nasal swab for MRSA.
13. Induce and collect sputum for bacterial culture, antibiotic sensitivity, MRSA colony forming units, small colony variants of MRSA, MRSA comprehensive genetic strain analysis and another cup for sputum cell count.
14. Schedule subject for Visit 6 in 60 days (± 7 days)

10.11 Visit 6 Day 118 (± 7 days) (3 Months Post-treatment Monitoring Visit)

1. Perform CFQ-R.
2. Record follow-up medical history.
3. Record any adverse events.
4. Record changes to concomitant medications.
5. Perform complete physical examination.
6. Perform and record vital signs including height and weight.
7. Perform and record oximetry.
8. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
9. Perform and record spirometry.
10. Induce and collect sputum for bacterial culture, antibiotic sensitivity, small colony variants of MRSA, and MRSA comprehensive genetic strain analysis.

10.12 Early Withdrawal Visit (for any subject terminating study early and after Day 1)

1. Perform CFQ-R.
2. Record follow-up medical history.
3. Record any adverse events.
4. Record changes to concomitant medications.
5. Collect all used and unused study medication and containers and Subject Diary (if applicable).
6. Review subject diary for adverse events and dosing adherence.

7. Perform complete physical examination.
8. Perform and record vital signs including height and weight.
9. Perform and record oximetry.
10. Collect blood sample for clinical laboratory tests: Chemistry, LFTs, Hematology, vancomycin level, and CRP if the visit occurs in treatment period between Visits 1 and 4.
11. Patient takes two inhalations of albuterol HFA 10-30 minutes prior to spirometry.
12. Perform and record spirometry.
13. If the early withdrawal visit occurs between Visit 3 and Visit 5, induce and collect sputum for bacterial culture, antibiotic sensitivity, MRSA colony forming units, small colony variants of MRSA, and MRSA comprehensive genetic strain analysis.
14. Collect all used and unused study medication and containers and Subject Diary.

11 ADVERSE EVENTS REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF beginning after the consent form is signed and at each visit until the last study visit. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study medication, or if unrelated, the cause. AEs will be followed until resolution.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0, as modified for cystic fibrosis, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 11-1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 11-1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 11-2.

Table 11- 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

The investigator will classify the study drug action taken with regard to the adverse event in the appropriate section of the eCRF. The action taken should be classified according to the categories shown in Table 11-3.

Table 11-3 Classifications for Study Drug Action Taken with Regard to an Adverse Event

Drug Interrupted	Study drug administration stopped in response to an adverse event.
Drug Withdrawn	Study drug administration permanently discontinued in response to an adverse event.
Dose Not Changed	Study drug dose not changed in response to the adverse event.
Dose Reduced	Study drug dose reduced in response to an adverse event.
Not Applicable	Action taken regarding study drug administration does not apply. "Not applicable" should be used in circumstances such as when the investigational treatment had been completed before the adverse event began and no opportunity to decide whether to continue, interrupt, or withdraw treatment is possible.

Adverse Event Outcome

An adverse event should be followed until resolution. The investigator will describe the outcome of the adverse event in the appropriate section of the CRF. The outcome should be classified according to the categories shown in Table 11-4.

Table 11-3 Classifications for Outcome of an Adverse Event

Recovered/Resolved	Resolution of an adverse event with no residual signs or symptoms.
Recovered/ Resolved With Sequelae	Resolution of an adverse event with residual signs or symptoms.
Not Recovered/ Not Resolved	Either incomplete improvement or no improvement of an adverse event, such that it remains ongoing.
Fatal	Outcome of an adverse event is death. "Fatal" should be used when death is at least possibly related to the adverse event.
Unknown	Outcome of an adverse event is not known, e.g., a subject lost to follow-up.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

All serious adverse events (SAEs), as defined below will be reviewed by the site investigator, recorded on an SAE Reporting form and followed through to resolution by a study physician. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed. All SAEs will be reported by telephone, email or fax within 24 hours (one working day) of awareness of the event to the following:

Cystic Fibrosis Foundation TDN DMC Chairman: Dr. Wayne Morgan, phone: (520) 626-6754 fax: (520) 626-9465, or email: wmorgan@arc.arizona.edu

Also, in accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the local IRB/IEC.

At Johns Hopkins this will be:

Johns Hopkins Medical Institutional Review Board, Reed Hall B-130; 1620 McElderry Street, Baltimore, Maryland 21205-1911. Phone: 410-955-3008, Fax: 410-955-4367 or 443-287-5353, or email: jhmirb@jhmi.edu

At Case University this will be:

University Hospitals Case Medical Center Institutional Review Board
Lakeside Hospital Rm. 1400
11100 Euclid Avenue
Cleveland, Ohio 44106
Phone: (216) 844-1529
FAX: (216) 844-1547
http://www.uhhospitals.org/Research/InstitutionalReviewBoard/tabid/1294/Default.aspx

A written SAE report must follow as soon as possible, which includes a full description of the event and any sequelae. The site will notify the TDN DMC of additional information or follow-up to an initial SAE Report as soon as relevant information is available.

A serious adverse event is any event that:

- is fatal
- is life-threatening (life-threatening is defined as the subject was at immediate risk of death from the AE as it occurred)
- is significantly or permanently disabling
- requires hospitalization, or prolongs hospitalization
- is a congenital anomaly or birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug experiences when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse. All SAEs will be followed until resolution including in any participants removed from the study due to toxicity.

11.2.2 Reporting SAEs to the FDA

The PI will use the following determinations to report SAEs to the FDA:

Does the reported adverse event meet one of the FDA's or the protocol's definitions for serious?

Is the event unexpected?

Is the event related to study drug?

If the answer to all 3 questions is yes, the event will be reported to the FDA in addition to all other relevant regulatory authorities as discussed in Section 11.2.1 of this protocol. The SAE form will be submitted to the FDA within 10 working days of the initial report.

11.3 Known Potential Risks of Study Drugs/Procedures and Specific AE Guidance

11.3.1 Inhaled Albuterol

Inhaled albuterol will be taken prior to inhalation of study drug/placebo. Albuterol may cause tremor, nervousness, and tachycardia. All of the individuals in the trial will have been exposed to albuterol in the past as part of their clinical care so will be aware of potential albuterol effects

11.3.2 Nebulized Vancomycin

The main risk of nebulized vancomycin is bronchospasm, although the incidence across several series of reports is less than 5% (Appendix 2). To minimize the risk of bronchospasm, participants will be pretreated with two puffs of inhaled albuterol HFA inhaler 10-60 minutes prior to administration of nebulized vancomycin. Patient will then do spirometry and have continuous pulse oximetry tested, followed by administration of nebulized vancomycin or placebo. Immediately after nebulizing the study drug, and again 15 (+/-10) minutes after completing the nebulization, participant will repeat spirometry. Previous studies of nebulized colistin, a commonly used and generally well-tolerated CF medication have suggested that inhalation is associated with an average drop in FEV_1 of 13±8% in patients without an asthma history and 17±8% in patients with a history of asthma[25]. The guidelines for spirometry after nebulized vancomycin are therefore:

1) FEV_1 percent predicted improves, is stable, or has a relative decrease less than 10% from baseline: patient considered tolerating nebulization and protocol proceeds.

2) FEV1 percent predicted has a relative decrease of 10-20% from baseline: All participants are monitored with appropriate follow-up treatment including PFTs until FEV1 has returned to within 10% of baseline. If a participant is symptomatic (chest tightness or dyspnea), the participant is treated immediately with two puffs of inhaled albuterol HFA inhaler. If a participant remains asymptomatic but has not returned to within 10% of baseline within 10 minutes of spirometry initiation, the participant is treated with two puffs of inhaled albuterol HFA inhaler. If patient does not note dyspnea and returns to within 10% of baseline within one hour of inhalation, patient may participate in the study. If FEV does not return to 10% of baseline after an hour, the participant is withdrawn from the study regardless of symptoms.

3) FEV_1 percent predicted has a relative decrease of 20% or more from baseline: Participant is treated immediately with two puffs of inhaled albuterol HFA inhaler and continually monitored with appropriate follow-up treatment until FEV_1 has returned to within 10% of baseline. Patient is withdrawn from the study.

A second potential risk is the development of vancomycin resistant strains of MRSA. Given that patients will also be treated in the protocol with other antibiotics to which their MRSA is known to be sensitive, this concern is minimized. This emergence of resistant MRSA has not been reported in any inhaled vancomycin studies to date. Finally, there is the potential emergence of gram negative pathogens as observed with the prophylaxis of MSSA in previous treatment trials. However, because most patients with MRSA are also co-infected with *P. aeruginosa* (70-80%) emergence of *P. aeruginosa* will not be a concern for a large number of patients. Other potential risks of nebulized vancomycin include bad taste and idiosyncratic allergic reaction.

11.3.3 Nebulized Placebo

The main risk of inhaling the nebulized placebo is experiencing a mildly bitter taste. There is little known risk for inhalation of sterile water. The quinine dose used is less than half that used in placebo in previous well tolerated nebulized drug trials.

11.3.4 Oral Trimethoprim/Sulfamethoxazole (TMP/SMX)

The main risk of taking TMP/SMX is an allergic reaction in those sensitive to sulfa products, usually manifested as a generalized rash. Most if not all of the participants will have received TMP/SMX in the past or be aware if they have a sulfa allergy. In those with known sulfa allergy, doxycycline will be substituted. Another potential reaction is nausea, although participants will be encouraged to avoid taking their oral medications on an empty stomach to minimize this risk.

11.3.5 Oral Doxycycline

Only individuals with known sulfa allergies will receive doxycycline. Oral doxycycline is generally well tolerated, with the main risks being nausea and rash.

11.3.6 Oral Rifampin

All individuals except those with a known allergy and/or intolerance, those individuals whose current CF treatment regimen includes medication(s) that are known to produce adverse drug-drug interactions with rifampin, or those individuals with evidence of rifampin resistance at Screening will receive oral rifampin. The most commonly observed potential side effect of rifampin is gastroesophageal reflux symptoms (GERD). For individuals experiencing this, an adverse event will be recorded and treatment will instituted by either increasing current GERD therapy or instituting treatment with a proton pump inhibitor. The proton pump inhibitor will be ordered by the patient's primary CF physician. Continued symptoms judged by the investigator or participant as more than mild despite treatment will result in discontinuation of the rifampin, although participants will be permitted to remain in the study. One other potential risk of oral rifampin includes elevated liver function tests. Patients will undergo laboratory monitoring at 2 and 4 weeks into their course.

11.3.7 Nasal Mupirocin Ointment

Nasal mupirocin is generally well tolerated, with no serious reactions reported related to short term use. Occasional participants may report localized burning at the site of nasal application.

11.3.8 Hibiclens Liquid Skin Cleanser

One of the identified potential side effects of Hibiclens Liquid Skin Cleaner with chlorhexidine is skin sensitivity, although the absolute risk is low and Hibiclens is currently routinely used in healthcare settings. Participants will undergo an observed test hand washing to assess for skin reaction prior to dispensing for home use. Participants that demonstrate significant rash or skin sensitivity to the Hibiclens Liquid Skin Cleanser will be allowed to participate in the protocol but will not do the skin washes with Hibiclens described in the protocol and will not be included in the data analysis of axillary culture results.

11.3.9 Sani-Cloth Alcohol Free Germicidal wipes

Sani-Cloth Alcohol Free Germicidal wipes were selected specifically because of the absence of odor. The main risk is skin irritation, so participants will be provided non-latex gloves for use and instructions on avoiding skin or eye contact.

11.3.10 Laboratory Work

Blood sampling can be associated with discomfort, fainting or lightheadedness and/or bruising. There is no known adverse event associated with the collection of urine specimens.

11.3.11 Sputum Induction Procedure and Spirometry

The risks associated with the sputum induction (SI) procedure consist of cough, wheeze, chest-tightness, and decrease in pulmonary function test values. These are generally mild in nature and self-limited, however, all subjects will be pre-treated with 2-3 puffs of albuterol prior to the SI procedure to minimize these risks. Subjects will fast one hour prior to the procedure. Pulmonary function is monitored frequently throughout the 12-minute procedure and the procedure is terminated if the peak flow decreases more than 20% from baseline.

Spirometry testing rarely causes discomfort however, some people get a sense of dizziness and/or headache when performing these tests.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Withdrawal of Subjects and Drop-outs

A subject may be discontinued from the study or study treatment at any time if the subject, the investigator, or the DSMB feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study or treatment discontinuation:

Subject withdrawal of consent

Subject is not compliant with study procedures

Adverse event

Lost to follow-up

At the discretion of the investigator, if deemed appropriate, for any reason

At the discretion of the DMC, if deemed appropriate, for any reason

If a subject is withdrawn from the study due to an adverse event any time after first dose of study drug, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized. Subjects will be encouraged to complete the early withdrawal visit.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. If a subject decides to withdraw after first dose of study drug on Day 1, the subject will be asked to return to the clinic to complete an Early Withdrawal Visit.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw from the study prior to day 58 (primary endpoint) will be replaced. Subjects who withdraw after day 58 will not be replaced. Refer to Section 10 for early termination procedures.

12.2 Trial Modification Rules

Given the early phase of the study and the small study sample size, establishing formal trial modification or stopping rules based on predefined statistical analyses is not possible. Determinations of safety require medical judgments of the DMC that cannot be prospectively defined in detail. The reasons for stopping, interrupting, modifying the trial, or changing the DMC safety monitoring plan could include but are not limited to:

• A pattern of SAEs that may possibly be drug related because they are consistent with the known effects of the drug; a clear temporal association with the use of the drug; improvement upon withdrawal of the drug; and/or lack of alternative explanations (e.g., concurrent disease or other drugs or chemicals).

• The occurrence of drug-related Grade 3 or Grade 4 adverse events or laboratory abnormalities in ≥ 33% of patients in a treatment arm.

• Unexpected increases in the incidence or severity of disease-related symptoms (e.g., cough, dyspnea, and/or decline in lung function (> 20% decline over the study treatment period of 1 month) that appears to be drug-related because there are not likely alternative explanations for these events

Specific SAEs which would result in termination of the study include:

1) A single respiratory event at any time that is judged by the DMC as life-threatening and probably related to study drug.

2) Greater than 20% absolute decline in FEV1 during the study drug treatment month in more than 33% of individuals and determined by the DMC to be probably related to study drug, any time after enrollment of the first 16 participants.

3) Bronchospasm with initial nebulization of study drug resulting in withdrawal from the study as described in protocol section 11.3.2 in more than 33% of individuals, any time after enrollment of the first 16 participants.

4) Trough serum vancomycin levels in excess of 8 ug/ml in more than 33% of individuals, any time after enrollment of the first 16 participants

5) Chemistry or hematologic abnormalities graded as level 3 or more in more than 33% of individuals and judged by the DMC as probably related to study drug, any time after enrollment of the first 16 participants

13 PROTOCOL VIOLATIONS

Protocol Violations for this study include, but are not limited to, the following: failure to meet inclusion/exclusion criteria, investigator non-compliance with study drug regimen, use of a prohibited concomitant medication, etc.

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The DSMB will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated by the investigator or monitor. This form will be signed by the Investigator. A copy of the form will be filed in the site's regulatory binder and submitted to Hospital IRB.

14 DATA SAFETY MONITORING

The Cystic Fibrosis Foundation Data Safety Monitoring Board (DSMB) will establish a Data Monitoring Committee (DMC) to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study, according to the Cystic Fibrosis Foundation Data Safety Monitoring Board Operations Manual and a DMC Charter to be established for this protocol. After 16 adult participants have been enrolled, the DMC will conduct a review for the purpose of monitoring study conduct and assessing patient safety. A follow up review will occur after the first 8 pediatric participants have been dosed and/or six months following IRB approvals to enroll pediatric patients, whichever comes first. Further details regarding the timing and content of the interim reviews is included in the statistical section below.

All participant withdrawals due to respiratory adverse events will be reported to the DSMB chair within 48 hours of the study team being aware of the event.

The DSMB will receive monthly reports to include:

Enrollment for both groups: 12-17 years and 18-years and up
Screen failures or study withdrawals
All FEV1 follow-up data after administration of study drug

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive at least one dose of the study drug (the Safety Population) will be included in the safety analyses.

15.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by treatment arm: race, gender, age, height and weight.

15.3 Analysis of Primary Endpoint

Our hypothesis for our primary outcome states that the treatment arm will result in significantly greater eradication of persistent MRSA from the respiratory tract of CF adolescents and adults compared to the placebo arm on day 58. Our primary analysis will include comparing the proportion of CF patients in the treatment arm who have a negative MRSA culture at Visit 5 to the proportion of patients in the placebo arm who have a negative MRSA culture at Visit 5. Secondary analyses will include the above comparisons, but at Visits 4 and 6. χ^2 or Fishers exact test will be used to determine statistical significance. A secondary analysis will also be performed comparing proportions of MRSA positive cultures among the three cultures obtained at Visits 3, 4, and 5 during the study. Generalized estimating equations (GEE), using a logit link, will be used to model this longitudinal data. The GEE model will be used to test for main effects of treatment arm, time, and treatment x time interaction. Furthermore, sensitivity analyses will be performed to evaluate the robustness of the primary outcome to missing culture data (missing data should be minimal) using 1) imputation with last observation carried forward and multivariate imputation by chained equations 2) treating all missing cultures as negative, and 3) treating all missing cultures as positive.

15.4 Analysis of Secondary Endpoints

Our hypothesis for these secondary outcomes states that the treatment arm will improve clinical measures (lung function, QOL, and exacerbations) compared to placebo. Statistical significance for

differences in lung function (FEV_1%) at Visit 4 will be assessed between the two treatments using a 2-sample t-test. We will use linear mixed models to longitudinally analyze and compare the change in lung function over 208 days in the two arms. CF respiratory scores will be expressed as mean ± standard deviation and statistical significance assessed between the two arms at Visit 4 using a 2-sample t-test. A Kaplan-Meier plot will be used to graphically display estimates of the survivor function for the proportion of patients who do not have an exacerbation through day 118. Hazard ratios and 95% CI due to treatment will be calculated using a Cox proportional hazards regression model[26]. Pre-specified potential variables that may be adjusted for include gender, season of enrollment, and center.

Hypothesis (H_{1c}): Our third hypothesis is that the treatment will have similar effects in regards to the development of antimicrobial resistance and adverse events compared to placebo. Previous MSSA treatment studies have resulted in increased gram negative pathogens; therefore, we will monitor for this development. Rates of occurrence of development of microbial resistance, gram negative pathogens will be summarized separately by treatment arm. Between-group comparisons will be made using a chi-square test or Fishers exact test.

Safety and tolerability data will be summarized by treatment group.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. Fisher's exact test will be used to compare the various treatment groups with respect to incidence of the more commonly occurring AEs.

15.5 Interim Analysis

No interim analyses are planned. A safety analysis will be planned after the first 16 adults are enrolled. If the DSMB agrees based on initial safety data, enrollment eligibility will then be expanded to include those aged 12-17 as well. A follow-up safety analysis will be performed by the DSMB after the first 8 pediatric participants have been dosed and/or six months following IRB approvals to enroll pediatric patients, whichever comes first.

15.6 Sample Size and Randomization

This study is powered on the primary outcome: the percentage of patients that are induced sputum negative for MRSA at Visit 5 (day 58). We are planning a study with 20 experimental and 20 control patients. There are no treatment studies published in CF patients with persistent MRSA to guide the assumptions for the primary outcome for the two arms. In fact, the results from this study will be used to power future multi-center trials. Based on our experience, treatment with oral antibiotics in those with persistent MRSA results in an approximate eradication rate of 15-25%. An experimental arm eradication rate of 75% would be considered significant, though some may even consider a lower percentage significant as well. Based on these assumptions, we have greater than 80% power if the MRSA eradication rate in the experimental arm is at least 65%, assuming a 20% eradication rate in the placebo arm (multiple assumptions about the eradication rate and their associated power are shown in Table 1). Ultimately, the eradication rate that is deemed clinically significant will be measured against the potential negatives of an intense treatment protocol including side effects and increased treatment burden.

Table 1: Power Estimates for Three Different Assumptions of Percent Eradicated in High Intensity Treatment Arm

Percent eradicated (Experimental Arm)	80%	75%	65%
Power	0.98	0.95	0.84
N = 40 Persistent MRSA patients (20 in each arm); two tailed α = 0.05; Control arm eradication rate: 20%			

Randomization will be performed by a biostatistician at Case in cooperation with each centers investigational drug service, with 1:1 randomization to placebo and study drug. The randomization will be designed to assure an equal division between placebo and treatment among the first sixteen participants in preparation for the first DSMB safety review (after 16 participants are enrolled).

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

Source documents will be created for this protocol and an electronic data base will be created which will serve as the case report forms (CRFs) for the research staff. Secondarily, information derived from the source documents/CRFs and required for the DMC reports will be entered into a spreadsheet for the purpose of data analysis. The spreadsheet may include, but is not limited to: demographic information, adverse events including seriousness, severity, relatedness to study drug or procedures, medical history, laboratory results and concomitant medications. Study personnel may enter data from source documents onto case report forms and the DMC spreadsheet. The spreadsheet will be secured and password protected. Study participants will not be identified by name in the study spreadsheet or on any source documents/CRFs but will be identified by a site number, subject number and initials. All clinical information requested in this protocol will be entered on the CRFs. If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved query reports. All changes to the study database will be documented.

16.4 Archiving of Data

At all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access. Databases are backed up by the database administrator in conjunction with any updates or changes to the database. Backup copies will be maintained at an off-site safe storage location. When the structure of the database is changed, a permanent archive of the database will be made to protect against loss of data in the changeover. When each backup is made, the media will be checked for usability and the integrity of the database will be verified.

At critical junctures of the protocol (e.g., production of interim reports and final reports), a permanent archive of the database will be made. Archived versions of the database will be saved for at least three years after the end of the study.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the DSMB (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and

copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of 2 years following completion of the trial.

16.6 Monitoring

Each site will hire an independent monitor who will monitor the study in accordance with current GCPs. On site – checking of the eCRFs for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. By signing this protocol, the Investigator grants permission to the monitor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the DSMB.

17 ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials and no patient names. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the principal investigator or his designee. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse events regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain a list of IRB/IEC members or other assurance of compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse events occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form and Recruitment Plan

Participants will be recruited from amongst the adult and pediatric CF patients at each of the two participating centers. The investigators and sub-investigators on this protocol will have access to these patients because they are the primary caregivers for the patients. Potential participants will be approached in CF clinic by one of the investigators, clinical nurses or research nurses. This will take place in the privacy of the exam room either while they are waiting to be seen by their physician or after they have been seen. Potential subjects will be provided information verbally about the study and if interested, they will be provided with contact information for the study team and a consent form to take home and review.

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

An IRB-approved copy of the Informed Consent Form will be kept in the Regulatory Binder.

The Informed Consent Form will document the study-specific information the Investigator provides to the subject and the subject's agreement to participate, including information about the Health Insurance Portability & Accountability Act (HIPAA) that protects the subject's individually identifiable health information (protected health information) and authorization (or agreement) in order for researchers to be able to use or disclose the subject's protected health information for research purposes. Among other things, the Investigator will fully explain in layman's terms the nature of the study, along with the aims, methods, anticipated benefits, potential risks and discomforts, and any monetary compensation participation may entail. The Informed Consent Form must be signed and dated before any study procedures are performed. The original and any amended signed and dated Informed Consent Form(s) must be retained in the subject's file at the study site; and a copy must be given to the subject.

17.4 Participant Risk / Benefit Assessment

Overall the Risk / Benefit Balance in this study is heavily in favor of potential benefit. MRSA is the most rapidly increasing infectious pathogen seen in individuals with CF, with 25% of patients now demonstrating pulmonary infection with MRSA[3]. The current therapeutic strategies for eliminating MRSA infection have been ineffective and little clinical trial data is available to guide therapy. Small clinical studies suggest that the addition of nebulized to vancomycin to current regimens improves clinical outcomes[11;12;14;15].

The risks involved with this protocol mainly involve those related to nebulized vancomycin. The other drugs utilized in the treatment regimen and the study procedures are all routinely used in clinical practice and clinical research. The main potential risk from nebulized vancomycin is bronchospasm. While nebulized vancomycin is commonly used clinically and has been well tolerated, there have been reports of mild bronchospasm. Review of studies and experience to date suggest the incidence to be <5% (Appendix 2)[12]. To minimize the risk of bronchospasm, patients will nebulize or inhale albuterol prior to their inhaled vancomycin dose. At Visit 1, participants will be monitored closely while nebulizing vancomycin/placebo for the first time, including pre and post nebulization spirometry to assure tolerance of study drug prior to having study drug dispensed for home use. This witnessed study drug nebulization is repeated within 2-7 days (Visit 2) to provide additional participant monitoring. Other potential risks of use of nebulized vancomycin include development of vancomycin resistant MRSA (unlikely given the short term use) and idiosyncratic allergic reaction. These are discussed in more detail in section 11.3.2. Most importantly however, current evidence suggests that the potential for side effects

from the change in route of administration of vancomycin to nebulization is actually significantly less than the risks seen with administration of intravenous vancomycin (which requires close serum monitoring and is known to be associated with renal toxicity and ototoxicity).

The identified risks of nebulized vancomycin are also well balanced by the potential benefits of MRSA eradication, which include both immediate improved lung function and long term improvement in survival[4;5]. Persistent MRSA infection has also been identified as a risk factor for persistent lung function decline after CF pulmonary exacerbations and this risk for failure to recover with exacerbation would be eliminated[27].

17.5 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the Principal Investigator and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.6 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol, except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the IRB any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection by the IRB and DSMB (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1 – SCHEDULE OF STUDY VISITS

	SCREENING VISIT (Day-28) (± 2 days)	RUN-IN VISIT (Day -14) (± 4 days)	VISIT 1 (START TREATMENT) (Day 1)	VISIT 2 (REPEAT ADMIN) (Day 2-7)	PHONE VISIT 1 (Day 7-14)	VISIT 3 (MID-POINT TREATMENT) (Day 14) (± 2 days)	PHONE VISITS 2 & 3 (Day 14-21) (Day 21-28)	VISIT 4 (END TREATMENT) (Day 29) (±1 day)	VISIT 5 1 MONTH MONITORING (Day 58) (± 4 days)	VISIT 6 3 MONTH MONITORING (Day 118) (± 7 days)	EARLY WITHDRAW VISIT
Informed Consent	X										
Randomization		X[n]									
Medical History (a)	X[a]	X	X	X		X		X	X	X	X
Demographics	X										
Complete Physical Exam	X							X		X	X
Abbreviated Physical Exam		X	X	X		X			X		
Height, Weight	X	X	X	X		X		X	X	X	X
Vital Signs, Oximetry (b)	X	X	X[b]	X[b]		X[b]		X	X	X	X
CFQ-R (c)			X			X		X	X	X	X
Administer albuterol (d)	X	X	X	X		X		X	X	X	X
Spirometry (e)	X	X	X[e]	X[e]		X		X	X	X	X
Sputum Induction Procedure (f)	X	X				X		X	X	X	X[o]
Sputum Culture and Sensitivity	X	X				X		X	X	X	X[o]
Sputum MRSA Colony Forming Units (g)	X	X				X		X	X	X	X[o]
Small Colony Variants of MRSA (g)	X	X				X		X	X	X	X[o]
MRSA: Comprehensive Genetic Strain Analysis (g)	X	X				X		X	X	X	X[o]
Sputum Cell Count (h)		X				X		X	X		
Sputum Cytokine Measurements (h)		X						X			
Nasal, Axillary, Rectal Swabs for Culture (i)		X[i]						X[i]	X[i]		
Administration of Study Drug			X[b]	X[b]		X[b]					
Expectorated Sputum Vancomycin Level (j)			X								
Serum Vancomycin Levels (k)			X[k]			X[k]					X[q]

	SCREENING VISIT (Day-28) (± 2 days)	RUN-IN VISIT (Day -14) (± 4 days)	VISIT 1 (START TREATMENT) (Day 1)	VISIT 2 (REPEAT ADMIN) (Day 2-7)	PHONE VISIT 1 (Day 7-14)	VISIT 3 (MID-POINT TREATMENT) (Day 14) (± 2 days)	PHONE VISITS 2 & 3 (Day 14-21) (Day 21-28)	VISIT 4 (END TREATMENT) (Day 29) (±1 day)	VISIT 5 1 MONTH MONITORING (Day 58) (± 4 days)	VISIT 6 3 MONTH MONITORING (Day 118) (± 7 days)	EARLY WITHDRAW VISIT
Chemistry/LFTs/Hematology/CRP	X		X			X		X	X		X[p]
Pregnancy Test (Urine or Serum) (l)	X[l]		X[l]			X[l]					
Initiate Subject Diary		X									
Subject Diary Review			X	X	X	X	X	X	X		X[p]
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X
Adverse Experiences Review		X	X	X	X	X	X	X	X	X	X
Hibiclens Test Hand Washing (m)		X									
Provide Snack			X			X					
Administration of protocol determined oral antibiotics, nasal mupirocin and Hibiclens			X			X[r]					
Dispense supply of study drug, nebulizer, protocol determined oral antibiotics, mupirocin and Hibiclens and provide teaching			X								
Collect used and unused study drug and containers								X			X[p]

a) History of previous treatment for MRSA, number of MRSA cultures, and length of MRSA positivity at screening visit

b) Continuous pulse oximetry is performed during nebulization of study drug and for 5 minutes afterward at Visit 1, Visit 2 and Visit 3. **Observe for 1 hour post nebulized study drug at Visit 1 and Visit 2.**

c) On days CFQ-R is performed, it is done before any other interventions

d) Two puffs of Albuterol 10-30 minutes prior to spirometry. *Participants will not have further Albuterol prior to induced sputum unless it occurs more than 2 hours after previous Albuterol.

e) Single spirometry value also obtained immediately after study drug nebulization and again 15 minutes (±10) after study drug nebulization at Visit 1 and Visit 2.

f) Follow TDN Sputum Induction protocol 530.02 or 535.01 (per Nebulizer type); throat swab if inadequate sputum produced

g) Only if culture grows MRSA

h) Follow TDN Sputum Processing Protocol 508.02

i) Rectal swabs optional and only age ≥18; no rectal swab at Visit 5

j) Spontaneous expectorated sputum collected 5±4 minutes after nebulizing study drug. Gargle with 30cc of saline for ten seconds x3 prior to collection.

k) Peak drawn 60 ±10 minutes after end of nebulization at Visit 1; trough drawn with other blood tests prior to nebulization at Visit 3.

l) Serum pregnancy test done at screening, urine at other time points.

m) Wash with water and one packet of Hibiclens hand cleanser for 2 minutes. Skin reaction will be observed for 15 minutes after washing

n) Randomization will be a non-visit task on Day -4 (±3 days) once culture results from Run-in confirm presence of MRSA

o) Sputum induction done if Early Withdrawal Visit occurs between Visit 3 and Visit 5.

p) If applicable, only if Early Withdrawal occurs between Visit 1 and Visit 4

q) If applicable, only if Early Withdrawal Visit occurs before Visit 3

r) Only oral antibiotic at Visit 3 if subject hasn't already taken that day.

APPENDIX 2 – Previous Published and Clinical Experience with Nebulized Vancomycin

First Author	Year	CF Status	# Patients	Dose	Frequency	Treatment Length	Side Effects
Shirai	1995	No	51	125mg	4x DAY	7-120 days	Vancomycin not detectable in serum 2 hours after inhalation. No side effects were reported in any of the 51 patients.
Doe	2010	Yes	18 courses	200mg	4x DAY	5 days	3 patients with chest tightness
Solis	2003	Yes	12 (18 courses)	4mg/kg/dose	4x DAY	5 days	None reported
Weathers	1990	No	1	40mg	3x DAY	6 days	None Reported in 3 y/o child on chronic vent
Gradon	1992	No	1	120mg	4x DAY	4 days	Serum vancomycin level 30min after administration was 2.2mcg/mL
Kahata	1997	No	1	Not reported	Not reported	Not reported	Cough, fever, shortness of breath, eosinophilia, hypoxia, and elevated CRP 30 minutes after inhalation in bone marrow transplant candidate. Resolved with steroids.
Maiz	1998	Yes	1	250mg	2x DAY	17 months	No VRE (checked twice). No VISA (checked 5 times). Creatinine normal throughout study. No otoxicity. No increase in cough or wheezing during vancomycin administration.
Miller	2003	No	1	120mg	3x day	1 day	Patient with ventilator associated pneumonia. Sputum level of 2,352 mcg/mL. No side effects reported.
Hayes	2010	Yes (post-Tx)	1	250mg	2x DAY	6 months	Undetectable serum vancomycin levels at 2 hours (checked twice). No change in creatinine
Clinical Location	**Year**	**CF Status**	**# Patients**	**Dose**	**Frequency**	**Treatment Length**	**Side Effects**
Foundation Care*	2004-2010	Yes	42	250mg	2x DAY	varying	None reported
Foundation Care*	2004-2010	Yes	22	125mg	2x DAY	varying	None reported
Case	2005-2010	Yes	22	125-500mg	2x DAY	up to 24 months	No VRE, VRSA, or chest tightness
Akron	2010	Yes	9	125mg	2x DAY		1 bronchospasm resolved with albuterol pretreatment.

18.3 APPENDIX 3 – Pulmonary Exacerbation Guidelines

18.3.1 Definition of a pulmonary exacerbation

For this study, pulmonary exacerbation is defined as a change in antibiotic therapy (IV, inhaled, or oral) for any 4 or more of the following signs/symptoms:

- change in sputum
- new or increased hemoptysis
- increased cough
- increased dyspnea
- malaise, fatigue, or lethargy
- temperature above 38°C (equivalent to approximately 100.4°F)
- anorexia or weight loss
- sinus pain or tenderness
- change in sinus discharge
- change in physical examination of the chest
- decrease in pulmonary function by 10%
- radiographic changes indicative of pulmonary infection

18.3.2 Data Collected on each Exacerbation

The following information will be determined for protocol-defined pulmonary exacerbations:

- Number of pulmonary exacerbations
- Number of days with pulmonary exacerbations
- Time-to-first pulmonary exacerbation
- Number of pulmonary exacerbations requiring hospitalizations
- Number of days hospitalized for pulmonary exacerbations
- Time-to-first hospitalization for pulmonary exacerbation
- Number of pulmonary exacerbations requiring IV antibiotic therapy
- Number of days on IV antibiotic therapy for pulmonary exacerbations
- Time-to-first IV antibiotic therapy for pulmonary exacerbations

Figure 1

Example

HOSPIRA
MCPHERSON, KANSAS
CERTIFICATE OF ANALYSIS

Sterile Vancomycin Hydrochloride, USP, 1 g/vial	Lot: 871934

Product Codes:	6533H		List Number:	0409-6533-04-20	
			NDC Number:	0409-6533-01	
Monograph number:	V-0121-X	Manufacture Date:	3-27-10	Expiration Date:	MAR 2012

TEST	SPECIFICATION	DATE TEST PERFORMED	RESULTS
Description (Lyophilized)	White to tan caked powder=Pass	3-31-10	Pass
Description (Reconstituted)	Light to dark tan solution	3-31-10	light tan solution
IR Identification <197K> or <197A>	Conforms to RS	4-2-10	Conforms to RS
Reconstitution Time	NMT 3 minutes (180 seconds)	3-31-10	34 seconds
Constituted Solution <1> 1. Completeness and Clarity of solution 2. Particulate Matter (Visual)	Meets USP Specifications	3-31-10	Meets USP Specifications
Vancomycin Potency <81>	90.0-115.0%	4-6-10	100.7 %
Vancomycin Purity (anhydrous basis)	NLT 900 µg/mg	4-6-10	1059 µg/mg
Visible Absorbance @ 450 nm	Report result	4-2-10	0.0403
Chromatographic Purity			
1. Vancomycin B	NLT 80.0% Vancomycin B	4-9-10	88.6 %
2. Other Peaks	NMT 9.0% of any other individual peaks	4-9-10	2.2 %
Water <921>	NMT 5.0%	4-5-10	0.7 %
pH<791>	2.5-4.5	3-31-10	3.4
Uniformity of Dosage Units (Weight variation) <905>	Meets Specifications	4-9-10	Meets Specifications
Heavy Metals <231>	NMT 0.003% as Pb	4-12-10	NMT 0.003 % as Pb
Sterility <71>	Meets USP Specifications	4-1-10	Meets USP Specifications
Bacterial Endotoxins <85>	NMT 0.33 EU/mg Vancomycin HCl	4-1-10	< 0.16 EU/mg Vancomycin HCl
Particulate Matter <788>	Meets USP Specifications	4-14-10	Meets USP Specifications
Residual Solvents <467>	Meets USP Specifications	NA	Meets USP Specifications

Information entered by/Date:	[signature] 4-20-10	[signature] 4-20-10

This product has been manufactured and tested in current Good Manufacturing Practices (cGMP) facilities in accordance with appropriate regulations. This product meets applicable specifications, applicable Regulatory Submissions or Marketing Authorizations and, where appropriate, Compendial requirements as listed in the above referenced monograph. The undersigned certifies this to be a true representation of the results.

Certified By/ Date:	Virginia Grose 4-21-10		
Approved By/Date:	C A. Kongs 6-25-08	Approved By/Date:	J. Wyckstandt 6-26-08

FIGURE 2: COMPOUNDING RECORD

VANCO PK Study Compounding Formula Record

Formula Name and Strength or Concentration		Dosage Form
Vancomycin 250mg/5ml in Sterile Water		Neb Ampule
Formula Quantity	**Container Size and Type**	**Beyond Use Period**
8	5ml neb ampules	60 days
Compounding Equipment		**Date**
Syringe pump lot # 1307971 Baxa Non Coring Vented Needles lot # 763308 exp 2014-06 Dispensing Spike lot # 10F15 exp 2013-06 Neb Ampules DD 5ml syringe lot # 1306665		3/12/12 **Final Concentration & Volume** Vancomycin 250mg/5ml Sterile Water
Ingredient or Supply		**Quantity**
Vancomycin 1000mg vial		2
Lot #	10125 DD	Hospira
Exp. Date	1 oct 2013	
Sterile Water - ~~10ml~~ 1000 ml		1
Lot #	13-045-JT	Hospira
Exp. Date	1 jan 2014	

Compounding Directions and Procedures

Prepare in Clean Room using sterile technique.
Withdraw 20ml of Sterile Water from vials.
Fill each vial of Vancomycin with 20 ml of Sterile Water.
Shake each filled vial to thoroughly mix Vancomycin and Sterile Water.
Using a 5ml syringe and dispensing spike, withdraw 5ml from Vancomycin vial.
Fill sterile neb vials with 5 ml and heat seal.
Results in Vancomycin 250 mg/ 5 ml ampules.

Clean Room Temperature	Pharmacist	Pharmacy Tech
62° / 55%	CMH	EAS

Room Cleaned	Clean Room Positive Pressure			
3/12/12	(Yes)	0.07	No	ante room 0.04

Vancomycin
250 mg/ 5 ml Sterile Water
Lot Vastudy-120312 Exp 05/12/12

Pharmacist Initials: CMH
Date Label Approved: 3/12/12

FIGURE 3 : PREPARATION DESCRIPTION

Chemistry, Manufacturing, and Control Data

1 Drug Substance

1.1 Summary Description

Vancomycin hydrochloride is a tricyclic glycopeptides antibiotic obtained from *Amycolatopsis orientalis*. It is an off-white lyophilized powder that is oxygen sensitive. Vancomycin may contain hydrochloric acid or sodium hydroxide to adjust pH.

1.2 Name and address of manufacturer

Hospira, Inc.
Lake Forest, IL 60045 USA

1.3 General Methods of Preparation

All preparations of vancomycin are done in a sterile compounding room based on USP policies and procedures. Vancomycin is reconstituted by adding 20 ml of Sterile Water to a 1 gram vial of sterile vancomycin powder.

1.4 Drug Substance Stability

Sterile Vancomycin Hydrochloride, USP [package insert]. Lake Forest, IL: Hospira, Inc.; 2008
Sterile Water for Injection, USP [package insert]. Lake Forest, IL: Hospira, Inc.; 2010

2 Drug Product

2.1 Summary Description

This drug product is vancomycin 250 mg / 5 ml Sterile Water solution compounded in a USP <797> Class 100 clean room via aseptic techniques and packaged in tamper proof, individual single-dose plastic ampules. The compounded sterile preparation is intended for inhalation via a nebulizer device.

2.2 List of Components

2.2.1 Active Ingredient

Vancomycin Hydrochloride 1000 mg PF Vial

2.2.2 Inactive Ingredient

Sterile Water 1000 ml

2.2.3 Container System

The 5 ml solution will be prepared into plastic pre-sterilized 5 ml nebulizer ampules. A twenty eight (28) day supply of ampules will be counted by the pharmacy staff, labeled according to study protocol, and dispensed to the patient or study investigators in a container (box).

2.2.4 Quantitative Composition

Individual 5 ml ampules contain:

Vancomycin Hydrochloride	250 mg
Sterile Water	5 ml

2.3 Name and Address of the Compounder

Foundation Care LLC
4010 Wedgeway Court

Earth City, Missouri 63045

Foundation Care was an FDA inspected facility. Foundation Care is also Pharmacy Compounding Accreditation Board (PCAB) accredited (a voluntary accreditation program).

2.4 Description of the Manufacturing and Packaging Procedures

When materials arrive for use, they are recorded and stored in accordance with standard operating procedures. Vancomycin sterile powder is stored at USP controlled room temperature (20-25°C) and sterile water is stored under refrigeration.

Compounding is performed in accordance with USP <797> in a Class 100 clean room. The clean room is maintained under positive pressure and includes HEPA filtration units. Personnel have been trained and evaluated for proper garbing, hand-washing, gloving, and aseptic technique. There are systems and documentation procedures for the maintenance, clearing, calibration, and usage of the clean room and equipment. Only one drug substance is compounded at a time in the clean room to decrease risk for cross-contamination with other substances. All work areas and equipment in the clean room are sanitized according to Standard Operating Procedures before and after each compounding activity.

Unopened vials of sterile vancomycin powder will be disinfected with 91% isopropyl alcohol in the ante room. The vials will then be brought into the clean room where the dust covers are removed. Compounding equipment utilized includes: Baxa Repeater Pump, Baxa Repeater Pump tubing, Baxa non-coring vented needles, TPN Bag, and nebulizer ampules. The Baxa pump is prepared and a 1000 ml bag of Sterile Water solution is attached to the Baxa pump. The Baxa pump is then calibrated to deliver 20 ml of Sterile Water. Each vial of vancomycin powder is filled with 20 ml of Sterile Water using a Baxa non-coring vented needle. Each vial is then shaken gently to dissolve the powder. The Sterile Water is removed from the Baxa pump and the tubing is drained. An empty TPN bag is attached to collect the vancomycin solution using a new Baxa needle. The "Reverse" function of the Baxa pump is activated. The pump is set to withdraw vancomycin from each filled vial; the contents are withdrawn into the TPN bag. The TPN bag is removed and the Sterile Water bag is attached to calibrate the pump to deliver 5 ml. Once calibrated, the Sterile Water is removed from the Baxa pump and the tubing is drained. The TPN bag of vancomycin is then attached to the Baxa pump along with a 1.2 micron filter and Baxa needle. To test again for calibration, a syringe is filled to verify the 5 ml quantity set by the pump. The individual sterile nebulizer ampules are filled with 5 ml of solution and are then heat sealed. This results in vancomycin 250 mg / 5 ml filled ampules.

The finished product is reviewed before leaving the clean room (see section 2.5). Each batch is assigned an individual lot number and expiration date.

The sealed vancomycin ampules are labeled and stored in the refrigerator which is controlled between 2-8°C.

2.5 Product Testing and Stability Studies

Final Product Testing

The identity, strength, quality, and purity of the final product will be confirmed using a series of analytic assays. The compounding directions and procedures have been validated by prior analytical assay. The potency is ensured for each batch when following the same set of validated directions. Sample vials will be sent for testing from each batch to ensure sterility.

Before the drug product can leave the facility, pharmacy personnel perform a series of check and test in accordance with Standard Operating Procedures after compounding to ensure that the preparation is sterile and accurate. Each ampule is visually inspected for integrity. The product is visually inspected for appropriate solution color and solution volume. Each ampule is also checked for particulates, cloudiness, and container leakage before leaving the clean room; the product is double-checked before dispensing.

A sample vial from each batch is sent to test for sterility, fungal and endotoxins. A preliminary report is returned after 72 hours with a final report given after 14 day incubation period. These analytical assays will be conducted by Analytical Research Laboratories, Oklahoma City, OK.

Table 1. Analytical assay performed

Test	Test Method
Sterility	USP <71> Sterility Test
Fungal, yeast, mold	Test media incubated at 25°C

Stability Testing

Beyond use dating studies have been performed. The beyond use period has been set at 60 days based upon prior testing of potency by contract laboratory at 0, 30, 60, 90, and 120 days after preparation.

The finished product is stored at USP refrigeration temperature (2-8°C); temperatures are checked and recorded daily. Based on prior validation, the product is potent and stable for greater than 60 days when stored in the refrigerator between 2-8°C.

FIGURE 4: STERILITY ANALYSIS (EXAMPLE)

ANALYTICAL RESEARCH LABORATORIES
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Microbiology Report

CLIENT: Foundation Care Pharmacy

ARL #: 162094-01
LOT #: VA250-111116
DESCRIPTION: Vancomycin 250 mg/5 mL in sterile water
DATE RECEIVED: 11/17/2011
STORAGE: 2°C to 8°C (35.6°F to 46.4°F)
CONTAINER: 1 bullet

ANALYSIS	Limits	Results	Test Method	Date Tested
Sterility (*Preliminary*)	Sterile / Not Sterile	Sterile	USP 71	11/17/2011
Fungal	Sterile / Not Sterile	Sterile	MBI-114	11/17/2011

Sterility - An Initial report will be issued after approximately 72 hours of incubation. In accordance with the USP guidelines, the samples will be incubated for 14 days; if there is any change in the sample a supplemental report will be issued.

Fungal - An Initial report will be issued after 4-5 days of incubation. In accordance with the USP guidelines, the samples will be incubated for 14 days; if there is any change in the sample a supplemental report will be issued.

Endotoxin - To calculate the endotoxin limit use the following formulae: EL = K/M where K = tolerance limit (EU/kg) and M = Maximum dose/kg/hour or Maximum dose/kg
Parenteral: K is 5 EU/kg for any route of administration /Intrathecal: K is 0.2 EU/kg body weight)
Radiopharmaceutical parenteral: K is 175/V or Intrathecal radiopharmaceuticals: K is 14/V, where V is the maximum recommended dose in mL.
Dermal Application: K/M, where K = 5 EU/kg and M is the (maximum dose/m2/hour × 1.80 m2)/70 Kg.

Tiffany O. Hyde — 11/21/2011

Tiffany Hyde - Microbiologist — Date Reported

ARL Form QUF-078-V4 03/05/2010

Results reported above relate only to the sample that was tested.

Page 1 of 1

ANALYTICAL RESEARCH LABORATORIES
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Microbiology Report

CLIENT: Foundation Care Pharmacy

ARL #: 162094-01
LOT #: VA250-111116
DESCRIPTION: Vancomycin 250 mg/5 mL in sterile water
DATE RECEIVED: 11/17/2011
STORAGE: 2°C to 8°C (35.6°F to 46.4°F)
CONTAINER: 1 bullet

ANALYSIS	Limits	Results	Test Method	Date Tested
Sterility	Sterile / Not Sterile	Sterile	USP 71	11/17/2011
Fungal	Sterile / Not Sterile	Sterile	MBI-114	11/17/2011

Tiffany O. Hyde

Tiffany Hyde - Microbiologist

12/01/2011
Date Reported

Sterility - 14 day sterility report. In accordance with the USP guidelines, the samples will be incubated for 14 days.

Results reported above relate only to the sample that was tested.

Page 1 of 1

ARL Form QUF-078-V4 03/05/2010

FIGURE 5: STABILITY ANALYSES

ANALYTICAL RESEARCH LABORATORIES
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Certificate Of Analysis

CLIENT: Foundation Care Pharmacy

ARL #: 162095-01
LOT #: VA250-111116
DESCRIPTION: Vancomycin 250 mg/5 mL in sterile water
DATE RECEIVED: 11/17/2011
STORAGE: 2°C to 8°C (35.6°F to 46.4°F)
CONTAINER: 1 bullet in a clear bag (5 TOTAL)

Analyte / Specifications	Expected Amount	Units	Results	% Of EXP.	Test Method	Date Tested
Vancomycin Specifications = 90% - 110%	250	mg/5 mL	259.828	103.9%	HPLC	11/21/2011

Time = Day 1 of testing at pre-defined timepoints.

Alex Tang - Laboratory Supervisor

11/21/2011
Date Reported

ARL Form QUF-078-V4 03/05/2010

Results reported above relate only to the sample that was tested.

Page 1 of 1

ANALYTICAL RESEARCH LABORATORIES
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Certificate Of Analysis

CLIENT: Foundation Care Pharmacy

ARL #: 162096-01
LOT #: VA250-111116
DESCRIPTION: Vancomycin 250 mg/5 mL in sterile water
DATE RECEIVED: 11/17/2011
STORAGE: 2°C to 8°C (35.6°F to 46.4°F)
CONTAINER: 1 bullet in a clear bag (5 TOTAL)

Analyte / Specifications	Expected Amount	Units	Results	% Of EXP.	Test Method	Date Tested
Vancomycin Specifications = 90% - 110%	250	mg/5 mL	270.077	108.0%	HPLC	12/21/2011

Time = Day 30 of testing at pre-defined timepoints.

Alex Tang - Laboratory Supervisor

12/21/2011
Date Reported

ARL Form QUF-078-V4 03/05/2010

Results reported above relate only to the sample that was tested.

Page 1 of 1

ANALYTICAL RESEARCH LABORATORIES
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Certificate Of Analysis

CLIENT: Foundation Care Pharmacy

ARL #: 162097-01
LOT #: VA250-111116
DESCRIPTION: Vancomycin 250 mg/5 mL in sterile water
DATE RECEIVED: 11/17/2011
STORAGE: 2°C to 8°C (35.6°F to 46.4°F)
CONTAINER: 1 bullet in a clear bag (5 TOTAL)

Analyte / Specifications	Expected Amount	Units	Results	% Of EXP.	Test Method	Date Tested
Vancomycin Specifications = 90% - 110%	250	mg/5 mL	265.079	106.0%	HPLC	1/18/2012

Time = Day 60 of testing at pre-defined timepoints.

Alex Tang - Laboratory Supervisor

01/18/2012
Date Reported

ARL Form QUF-078-V4 03/05/2010

Results reported above relate only to the sample that was tested.

Page 1 of 1

ANALYTICAL RESEARCH LABORATORIES
840 RESEARCH PARKWAY, SUITE 546
OKLAHOMA CITY, OK 73104
PHONE (405) 271-1144
FAX (405) 271-1174

Certificate Of Analysis

CLIENT: Foundation Care Pharmacy
Mike Schultz
4010 Wedgeway Court
Earth City, MO 63045

ARL #: 162098-01
LOT #: VA250-111116
DESCRIPTION: Vancomycin 250 mg/5 mL in sterile water
DATE RECEIVED: 11/17/2011
STORAGE: 2°C to 8°C (35.6°F to 46.4°F)
CONTAINER: 1 bullet in a clear bag (5 TOTAL)

Analyte / Specifications	Expected Amount	Units	Results	% Of EXP.	Test Method	Date Tested
Vancomycin Specifications = 90% - 115%	250	mg/5 mL	269.52	107.8%	HPLC	2/19/2012

Time = Day 90 of testing at pre-defined timepoints.

Alex Tang - Laboratory Supervisor

02/20/2012
Date Reported

ARL Form QUF-078-V4 03/05/2010

Results reported above relate only to the sample that was tested.

Page 1 of 1

Daily Respiratory Symptom Diary for Cystic Fibrosis

Self-Report Version (ages 12+ years)

Christopher Goss, MD, MSc, Principal Investigator
Donald Patrick, PhD, MSPH, Co-Investigator
Todd Edwards, PhD, Project Manager
Bonnie Ramsey, MD, Co-Investigator
James Lymp, PhD, Co-Investigator
Pauline Cooper, BS, Research Coordinator

University Of Washington
Depts. of Pulmonary Medicine and Health Services

NOTE: Do not reproduce or distribute without the consent of the authors.

Cystic Fibrosis: Your Daily Experience

Instructions

- Complete this diary between 5:00 P.M. and when you go to bed each evening.
- Think carefully about **your experience with cystic fibrosis, specifically during the last 24 hours**, before responding to each question. The "last 24 hours" is the amount of time that has passed since the same time the previous day.
- **Please complete all the questions in one sitting.**

Study ID number _____	
What is today's date? *(write-in your answer)*:	_____, _____ 20____ MONTH DAY YEAR
What is the current time? *(write-in your answer, and circle "AM" (before noon) or "PM" (after noon):*	_____:_____ o' clock AM PM

Note: Below, "Night" is defined as the time from when you go to bed until the time you get up the next morning.

Because of your cystic fibrosis, during the last 24 hours:

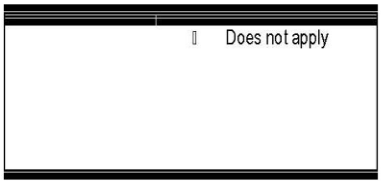
			During the last 24 hours, when did you..? *(Check one)*:	Overall during the last 24 hours, how much..? *(Check one)*:	Overall during the last 24 hours, how often..? *(Check one)*:
1. Did you have difficulty breathing?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #2 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
2. Did you feel feverish (have a temperature)?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #3 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
3. Did you feel tired?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #4 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
4. Did you have chills or sweats?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #5 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ Slightly ☐ Moderately ☐ Very ☐ Extremely	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
5. Did you cough?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #6 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ Slightly ☐ Moderately ☐ Very ☐ Extremely	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
6. Did you cough up mucus?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #7 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
7. Did you have tightness in the chest?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #8 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
8. Did you wheeze?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #9 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ Slightly ☐ Moderately ☐ Very ☐ Extremely	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time

Graphics Station:Users:donna:Desktop:Biomarker Study self-report diary one-page 5-1-07 - paper version.doc

Because of your cystic fibrosis, during the last 24 hours:

			During the last 24 hours, when did you..? *(Check one)*:	Overall during the last 24 hours, how much..? *(Check one)*:	Overall during the last 24 hours, how often..? *(Check one)*:
9. Did you have difficulty sleeping?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #10 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
10. Did you feel worried?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #11 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
11. Did you feel cranky?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #12 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
12. Did you feel sad or depressed?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #13 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
13. Did you feel frustrated?	*(Check one)*: ☐ Yes ☐ No	If No, GO TO #14 ▼	☐ Night only ☐ Day only ☐ Both day & night	☐ A little ☐ Somewhat ☐ A good deal ☐ A great deal	☐ A little bit of the time ☐ Some of the time ☐ Much of the time ☐ All of the time
14. How much time did you spend sitting or lying down?	*(Check one)*: ☐ Hardly any of the time ☐ Some of the time ☐ Most of the time ☐ All of the time				
15. Did you reduce your usual activities?	*(Check one)*: ☐ Yes ☐ No				
16. Did you miss work or school?	*(Check one)*: ☐ Yes ☐ No				

Graphics Station:Users:donna:Desktop:Biomarker Study self-report diary one-page 5-1-07 - paper version.doc



Are there any other symptoms you would like to report?

- ○ NO
- ○ YES:

_____

_____

If you were scheduled to take study drugs did you take them as instructed?

- ○ NO (details)_____
- ○ YES

- ○ Not scheduled to take study drug today

Graphics Station:Users:donna:Desktop:Biomarker Study self-report diary one-page 5-1-07 - paper version.doc

19. REFERENCE LIST

Reference List

1. Boyle, M. P. 2007. Adult cystic fibrosis. *JAMA* 298:1787-1793.

2. Dasenbrook, E. C. 2011. Cystic fibrosis and survival in patients with advanced lung disease. *BMJ* 342:d726.

3. Cystic Fibrosis Foundation, Patient Registry 2009 Annual Report. 2010. Bethesda, MD.

4. Dasenbrook, E. C., C. A. Merlo, M. Diener-West, N. Lechtzin, and M. P. Boyle. 2008. Persistent methicillin-resistant Staphylococcus aureus and rate of FEV1 decline in cystic fibrosis. *Am.J.Respir.Crit Care Med.* 178:814-821.

5. Dasenbrook, E. C., W. Checkley, C. A. Merlo, M. W. Konstan, N. Lechtzin, and M. P. Boyle. 2010. Association between respiratory tract methicillin-resistant Staphylococcus aureus and survival in cystic fibrosis. *JAMA* 303:2386-2392.

6. Gonzalez-Zorn, B., J. P. Senna, L. Fiette, S. Shorte, A. Testard, M. Chignard, P. Courvalin, and C. Grillot-Courvalin. 2005. Bacterial and host factors implicated in nasal carriage of methicillin-resistant Staphylococcus aureus in mice. *Infect.Immun.* 73:1847-1851.

7. Jesus Valle, M. J., F. G. Lopez, A. D. Hurle, and A. S. Navarro. 2007. Pulmonary versus systemic delivery of antibiotics: comparison of vancomycin dispositions in the isolated rat lung. *Antimicrob.Agents Chemother.* 51:3771-3774.

8. Garske, L. A., T. J. Kidd, R. Gan, J. P. Bunting, C. A. Franks, C. Coulter, P. J. Masel, and S. C. Bell. 2004. Rifampicin and sodium fusidate reduces the frequency of methicillin-resistant Staphylococcus aureus (MRSA) isolation in adults with cystic fibrosis and chronic MRSA infection. *J.Hosp.Infect.* 56:208-214.

9. Macfarlane, M., A. Leavy, J. McCaughan, R. Fair, and A. J. Reid. 2007. Successful decolonization of methicillin-resistant Staphylococcus aureus in paediatric patients with cystic fibrosis (CF) using a three-step protocol. *J.Hosp.Infect.* 65:231-236.

10. Solis, A., D. Brown, J. Hughes, H. K. Van Saene, and D. P. Heaf. 2003. Methicillin-resistant Staphylococcus aureus in children with cystic fibrosis: An eradication protocol. *Pediatr.Pulmonol.* 36:189-195.

11. Doe, S. J., A. McSorley, B. Isalska, A. M. Kearns, R. Bright-Thomas, A. L. Brennan, A. K. Webb, and A. M. Jones. 2010. Patient segregation and aggressive antibiotic eradication therapy can control methicillin-resistant Staphylococcus aureus at large cystic fibrosis centres. *J.Cyst.Fibros.* 9:104-109.

12. Generali, J. and D. Cada. 2004. Off-Label Drug Uses: Vancomycin: Aerolsolizaton. *Hospital Pharmacy* 39:638-647.

13. Shirai, M., K. Ide, M. Sato, M. Murakami, Y. Tanaka, A. Sato, and K. Chida. 1995. [Effect of inhaled vancomycin hydrochloride on elimination of methicillin-resistant Staphylococcus aureus]. *Nihon Kyobu Shikkan Gakkai Zasshi* 33:1233-1239.

14. Maiz, L., R. Canton, N. Mir, F. Baquero, and H. Escobar. 1998. Aerosolized vancomycin for the treatment of methicillin-resistant Staphylococcus aureus infection in cystic fibrosis. *Pediatr.Pulmonol.* 26:287-289.

15. Hayes, D., Jr., B. S. Murphy, T. W. Mullett, and D. J. Feola. 2010. Aerosolized vancomycin for the treatment of MRSA after lung transplantation. *Respirology.* 15:184-186.

16. Kahata, K., S. Hashino, M. Imamura, A. Mori, S. Kobayashi, and M. Asaka. 1997. Inhaled vancomycin-induced allergic reaction in decontamination of respiratory tracts for allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 20:1001-1003.

17. Miller, D. D., M. M. Amin, L. B. Palmer, A. R. Shah, and G. C. Smaldone. 2003. Aerosol delivery and modern mechanical ventilation: in vitro/in vivo evaluation. *Am J Respir.Crit Care Med.* 168:1205-1209.

18. Simor, A. E. and M. Loeb. 2004. The management of infection and colonization due to methicillin-resistant Staphylococcus aureus: A CIDS/CAMM position paper. *Can.J Infect.Dis.* 15:39-48.

19. Rose, W. E. and P. T. Poppens. 2009. Impact of biofilm on the in vitro activity of vancomycin alone and in combination with tigecycline and rifampicin against Staphylococcus aureus. *J Antimicrob.Chemother.* 63:485-488.

20. Goerke, C., K. Kraning, M. Stern, G. Doring, K. Botzenhart, and C. Wolz. 2000. Molecular epidemiology of community-acquired Staphylococcus aureus in families with and without cystic fibrosis patients. *J Infect.Dis* 181:984-989.

21. Mody, L., C. A. Kauffman, S. A. McNeil, A. T. Galecki, and S. F. Bradley. 2003. Mupirocin-based decolonization of Staphylococcus aureus carriers in residents of 2

long-term care facilities: a randomized, double-blind, placebo-controlled trial. *Clin.Infect.Dis* 37:1467-1474.

22. Stone, A., L. Quittell, J. Zhou, L. Alba, M. Bhat, J. Decelie-Germana, S. Rajan, L. Bonitz, J. J. Welter, A. J. Dozor, et al. 2009. Staphylococcus aureus Nasal Colonization Among Pediatric CF Patients and Their Household Contacts. *Pediatr Infect.Dis.J* 28:895-899.

23. McConeghy, K. W., D. J. Mikolich, and K. L. LaPlante. 2009. Agents for the decolonization of methicillin-resistant Staphylococcus aureus. *Pharmacotherapy* 29:263-280.

24. Quittner, A. L., A. C. Modi, C. Wainwright, K. Otto, J. Kirihara, and A. B. Montgomery. 2009. Determination of the minimal clinically important difference scores for the Cystic Fibrosis Questionnaire-Revised respiratory symptom scale in two populations of patients with cystic fibrosis and chronic Pseudomonas aeruginosa airway infection. *Chest* 135:1610-1618.

25. Alothman, G. A., B. Ho, M. M. Alsaadi, S. L. Ho, L. O'Drowsky, E. Louca, and A. L. Coates. 2005. Bronchial constriction and inhaled colistin in cystic fibrosis. *Chest* 127:522-529.

26. Cox, D. 1972. Regression models and life tables. *Journal of the Royal Statistical Society Series B-Statistical Methodology* 34:187-220.

27. Sanders, D. B., R. C. Bittner, M. Rosenfeld, L. R. Hoffman, G. J. Redding, and C. H. Goss. 2010. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. *Am J Respir.Crit Care Med.* 182:627-632.