Early MRSA Therapy in CF (Streamlined Eradication with Repeat Cycle) (STaph Aureus Resistance-Treat Early and Repeat (STAR-TER))

NCT number NCT03489629
Document Date 10/01/2020

Protocol Modifications:

1. Remote study visits in setting of COVID-19 precautions. This applies to patients who have completed visit 2 and have completed study medication i.e. those for visit 3 or visit 4. This change was implemented in April 2020 due to the coronavirus pandemic with an ongoing clinical trial based on site level IRB approval without a protocol modification. For patients meeting the above criteria, remote visits were allowed for visit 3 and 4. We now codify this change in a formal protocol modification given the continued pandemic. Sample collection will be done at home and mailed to the Core Microbiology lab at UNC.
2. Allow use of spirometry results from clinic visit +/- 2 weeks of the study visit. Spirometry in the research setting is has not been feasible for a number of our sites in the setting of COVID-19.

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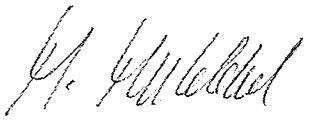
Clinical Research Protocol

Early MRSA therapy in CF (streamlined eradication with repeat cycle)

(**ST**aph **A**ureus **R**esistance-**T**reat **E**arly and **R**epeat (STAR-TER))

Protocol Number:	STAR-ter
Version Date:	October 1, 2020
Investigational Product:	Trimethoprim-Sulfamethoxazole, Minocycline
IND Number:	Not applicable
Development Phase:	Phase 2
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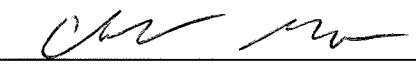


Oct 28, 2020

PI or Sponsor Signature (Name and Title)

Date

Approval:



Oct. 30, 2020

PI or Sponsor Signature (Name and Title)

Date

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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 Sponsor 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: STAR-TER 001

Protocol Title: **ST**aph **A**ureus **R**esistance-**T**reat **E**arly and **R**epeat (STAR-TER)))

Protocol Date: October 1, 2020

_____ _____

Investigator Signature *Date*

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LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CFRSD	Cystic Fibrosis Respiratory Symptom Diary
CFQ-R	Cystic Fibrosis Questionnaire – Revised
CFRSD_CRISS	Cystic Fibrosis Respiratory Symptom Diary-Chronic Respiratory Infection Symptom Score
CFTR	cystic fibrosis transmembrane conductance regulator
CF	cystic fibrosis
CLSI	Clinical and Laboratory Standards Institute
CRF	case report form
DMC	Data monitoring committee
DSMB	Data safety monitoring board
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in one second
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
MRSA	methicillin resistant *Staphylococcus aureus*
MSSA	methicillin susceptible *Staphylococcus aureus*
mEq	milliequivalent
PI	Principal Investigator
PVL	Panton-Valentine Leukocidin
SAE	serious adverse experience
SCC*mec*	Staphylococcal Cassette Chromosome *mec*
TDNCC	Therapeutics Development Network Coordinating Center
TMP/SMX	Trimethoprim sulfamethoxazole
ULN	Upper limit of normal

PROTOCOL SYNOPSIS

TITLE	**ST**aph **A**ureus **R**esistance-**T**reat **E**arly and **R**epeat (STAR-TER)
SPONSOR / Study PI	Marianne S. Muhlebach, M.D. and Christopher H. Goss, M.D., M.Sc.
FUNDING ORGANIZATION	Cystic Fibrosis Foundation
NUMBER OF SITES	8
RATIONALE	The prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) respiratory infection in CF has increased dramatically over the last decade with 26.0% of CF patients in the U.S. having a MRSA positive respiratory culture. Epidemiologic evidence suggests that persistent infection with MRSA may result in an increased rate loss of lung function as measured by the forced expiratory volume in 1 second (FEV_1) particularly in the pediatric population. Infection with MRSA has also been shown in an observational study to be associated with higher mortality, in those subjects with sustained MRSA positive cultures. These observations and higher medication and healthcare use after MRSA infection, warrant an early and aggressive approach to eradication of newly acquired MRSA. The well-documented difficulty in eradication of *Pseudomonas aeruginosa* once the infection has become chronic and the recent studies demonstrating effective eradication of that organism in early stages of the infection suggest that a similar approach may be appropriate for MRSA. Until recently, no comparative clinical trials have been done to assess the efficacy of MRSA eradication in early MRSA. The first randomized, controlled comparative trial of an aggressive MRSA eradication protocol to standard of care i.e. observation alone, has recently been completed = STAR-too (*Staph. Aureus* Resistance-treat or observe). The study demonstrated microbiologic efficacy of a comprehensive regimen involving two oral antibiotics, decolonization of mucus membranes and body wash combined with household cleaning as compared to no treatment. Specifically, after a one-month treatment protocol, there was a 56% absolute reduction in MRSA positive cultures as compared to the observational arm. Secondary endpoints showed trends towards better lung function and fewer antibiotic courses in those in the treatment arm. The results from the recent STAR-Too trial provide compelling data for the use of an aggressive multi-faceted treatment regimen to quickly convert a patient with a MRSA positive culture to culture negativity, thus prohibiting the use of placebo or observational control arms for future studies in this patient population. However, several additional questions remain to generalize and confirm the results in this trial and optimize the treatment strategy for feasibility and efficacy. Specific questions are if a

	simplified regimen would produce comparable microbiologic efficacy to the rigorous approach evaluated in the prior trial, and whether repeat cycles of therapy could enhance efficacy for patients who either are microbiologic failures after one month of treatment or to prevent re-occurrence in those who had responded initially. Cycled antibiotics are often used in CF. In the case of this protocol, a potential concern to monitor for would be emergence of resistance or small colony variants of *S. aureus*, a growth form of MRSA seen during chronic infection and after prolonged use of trimethoprim-sulfamethoxazole. The proposed study aims to address outstanding questions related to the optimization of early MRSA therapy in CF. As equipoise for the use of a control or observational group no longer exists, yet only incident cases are enrolled, this study will capitalize on the use of historical control data acquired during the recently completed STAR-too trial. This novel study design addresses the difficulty of implementing prospective controlled studies in rare patient populations. The *primary objective* of the study will be to assess if a more *streamlined treatment regimen will have comparable microbiologic efficacy* compared to the rigorous approach used previously. An exploratory outcome is to investigate if microbiologic efficacy can be enhanced safely with a repeat cycle, either to achieve eradication in those failing the first cycle or enhance effectiveness and prevent recurrence in those with initial.
STUDY DESIGN	This is an open-label, multi-center interventional trial in CF patients with new MRSA isolated from the respiratory tract (oropharyngeal = OP swab, sputum, or bronchoscopy) at a clinical encounter. 42 subjects with new MRSA infection will be enrolled and will receive two weeks of oral trimethoprim-sulfamethoxazole (TMP-SMX) for 14 days, and nasal mupirocin for 5 days. Subjects old enough to do so will use oral disinfectant gurgle (0.12% chlorhexidine gluconate oral rinse) for 14 days. The primary endpoint will be the proportion of positive MRSA respiratory cultures at Day 28 and this will be compared to our prior STAR-Too results. Subjects will then have a 14 day wash-out period (i.e. no TMP-SMX from Day 14 to Day 28) and all participants will repeat the treatment protocol from Day 29 to Day 42. Repeat cultures will be done at day 56 ± 7 days, most likely combined with their next clinic visit. Results of Day 56 cultures will be an exploratory, secondary outcome. Subsequent visits will be 3 and 6 months later with their routine clinic appointments. Any interim clinic visits will be used to obtain repeat culture and clinical data. Assessment of MRSA culture status will be by OP swab for all subjects, with additional sputum in those who expectorate. Contemporaneous Clinical Care Comparison Arm: Subjects with incident

	MRSA who meet eligibility criteria but are not interested in a protocolized study will be offered an opportunity to be part of the clinical care arm. Clinical data (MRSA culture status, antibiotic treatments, lung function etc.) will be collected for exploratory analyses comparing clinical outcomes of MRSA management outside the confines of a protocolized trial. Due to COVID-19 changes for patients who have completed visit 2, and have completed study medication i.e. those for visit 3 or visit 4. Patients may complete remote visits for visit 3 and visit 4 if the site does not have clinic access for the subject or coming to the CTRC or healthcare facility poses a risk (infection with COVID 19). This remote visit will involve: 1. Call the family 1-2 weeks prior to the planned visit to discuss the remote visit. 2. Mail swabs for an OP-swab on the study subject and for household contacts nasal swabs. We will pre-label the swabs with the study IDs so the core study lab can process it but attach a separate label for the family in plain text e.g. "OP CF" "Mom nose", "brother nose" etc. Mailing supplies appropriate for biological hazardous material for sending those back directly to the Core study lab at UNC will be sent to families. 3) The day of study visit we plan to call the family and review the questionnaires with them and fill all of the history and CF Quality of Life (CFQL) questionnaires with them over the phone. As well as update case report forms. In other words, the visit will be conducted remotely. 3a) Review technique of OP swab with them and ask the caregiver to perform the OP swab on their child. The visit can also be conducted by Zoom which is considered HIPAA compliant.
PRIMARY OBJECTIVE	To evaluate the microbiologic efficacy and safety of a streamlined two week MRSA eradication treatment protocol.

SECONDARY OBJECTIVES	1. Evaluate adherence to the MRSA eradication treatment protocol 2. Preliminary data on clinical outcomes (FEV_1 and Weight) 3. Assess the microbiologic efficacy and safety of repeated treatment, in regards to achieving culture negative status in those failing the first cycle or reduction of recurrence in those subjects who are MRSA negative after the first treatment course. Although there will be few subjects this can be compared to the initial trial where many subjects received antibiotics after Day 28. 4. Assess treatment burden and side effects of this streamlined protocol compared to the prior dual-antibiotic treatment. 5. Compare those participating in the study to those non-participants willing to consent to having their clinical data collected during routine clinic encounters. 6. Monitor for emergence of drug resistances or small colony variants after the first or second treatment cycle.
NUMBER OF SUBJECTS	38 completed. Anticipating a 10% attrition, planned enrollment will be 42 subjects.
SUBJECT SELECTION CRITERIA	**<u>Inclusion Criteria</u>:** 1. Male or female ≥ 2 and ≤ 45 years of age at the Screening Visit. 2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria: a. sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT) b. two well-characterized mutations in the cystic fibrosis transmembrane conductive regulator (CFTR) gene c. Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproteronol of less than -5 mV) 3. First **OR** early MRSA colonization defined as: a. <u>First MRSA colonization</u>: first life-time documented isolation of MRSA from respiratory tract. Occurrence no longer than 6 months prior to screening **OR** b. <u>Early MRSA colonization</u>: ▪ MRSA was previously isolated from the respiratory tract ≤ 2 times over the past 3.5 years, but this was followed by at least 1 year of documented

negative cultures for MRSA as noted below:

- At least 2 cultures performed a minimum of 3 months apart to document 1 year of culture negativity. Each of these cultures should be documented to have been collected at least 1 week after end of any antibiotic prescription with MRSA activity.
- Patient again recently positive for MRSA from the respiratory tract (within 6 months prior to screening)

4. MRSA isolate is available for testing in central lab – either isolated at the screening visit or available from the most recent clinic visit that qualified the subject as eligible
5. Written informed consent (and assent when applicable) obtained from subject's legal representative and ability for subject/family to comply with the requirements of the study

Exclusion Criteria:

1. Received antibiotics with activity against MRSA within 28 days prior to screening (listed in Appendix 1)
2. Requirement for oral or IV antibiotics, as determined by treating MD, at time of MRSA isolation
3. Use of an investigational agent within 28 days prior to screening
4. For subjects ≥ 6 years of age: FEV_1 at last clinic visit or at screening < 25% of predicted for age based on the Wang (males < 18 years, females < 16 years) or Hankinson (females ≥ 16 years) standardized equations
5. MRSA from the screening culture or the most recent clinical care visit prior to screening is resistant to TMP/SMX
6. History of intolerance to topical chlorhexidine or mupirocin
7. History of intolerance to **both** TMP/SMX and minocycline
8. < 8 years of age **and** allergic or intolerant to TMP/SMX
9. ≥ 8 years of age **and** allergic or intolerant to TMP/SMX **and** MRSA isolate (from screening or clinical visit) is resistant to minocycline
10. Subjects with history of abnormal renal function will need screening labs showing normal function Abnormal renal function is defined as estimated creatinine clearance <50 mL/min using the
    - Bedside Schwartz Equation for subjects <18 years of age, and

	• Levey GFR MDRD Equation for subjects ≥ 18 years of age. 11. Subjects with a history of abnormal liver function will need to have screening labs showing normal transaminases. Liver dysfunction is defined as ≥3x upper limit of normal (ULN), of serum aspartate transaminase (AST) or serum alanine transaminase (ALT) or abnormal synthetic function 12. History of solid organ or hematological transplantation 13. For females of child bearing potential: pregnant, breastfeeding, or unwilling to use barrier contraception through Day 42 of the study 14. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	**Day 1 – Day 28 and repeat cycle Day 29-Day 56** **Oral Antibiotics** 1) Trimethoprim/Sulfamethoxazole (TMP/SMX): a. Dosing if <40kg: 8 mg/kg trimethoprim / 40 mg/kg sulfamethoxazole) given **twice daily for 14 days.** Dose of trimethoprim may be rounded to a maximum of 10 mg/kg/dose for ease of administration; b. ≥ 40 kg: **320mg/1600 mg twice daily for 14 days** 2) If a subject has an allergy or intolerance to TMP/SMX, they may be treated with minocycline provided they are 8 years of age or older a. Dosing if < 50 kg: 2 mg/kg orally **twice daily for 14 days** b. ≥ 50 kg: **100 mg twice daily for 14 days** **Topical Antibiotics** 3) Mupirocin 2% nasal ointment applied to each nostril **twice daily for first 5 days** 4) For subjects able to gurgle without swallowing: 0.12% chlorhexidine gluconate oral rinse **twice daily for 14 days** **Attention to correct cleaning of nebulizers and airway clearance devices will be emphasized with handout of CFF guidelines**
DURATION OF SUBJECT PARTICIPATION	Subjects will be in the study for up to 6 months of which 56 days will be active treatment/washout. Observational follow-up Day 57 to Day 180. **Screening (V1):** up to 14 days prior to starting antibiotics on Day 1

AND DURATION OF STUDY	**Eradication Treatment Protocol:** 56 days **Observational Follow-up:** All subjects will be seen at each of their clinic visits and any sick visits.
CONCOMMITANT MEDICATIONS	**Allowed:** Standard therapy for CF is allowed except for treatments noted in the prohibited medications section below. Ongoing chronic treatment (>21 days prior to screening visit) with Pulmozyme®, Cayston®, high dose inhaled tobramycin, nebulized colistin, high dose ibuprofen, hypertonic saline, azithromycin, systemic steroids, or inhaled steroids, short and long bronchodilators and airway clearance is allowed. CFTR specific medications (Ivacaftor, Ivacaftor/lumicaftor, or FDA approved CFTR modulator) are allowed if those have been ongoing for 3 months prior. A stable therapeutic regimen between the screening visit and Day 56 is the goal. Unless otherwise medically indicated, subjects not using these therapies should not be started on them after screening or at any time prior to Visit 3 (Day 56) and subjects who have been using them chronically should be encouraged to continue them through Visit 3 (Day 56) (as per their scheduled dosing). After Visit 3 (Day 56) there are no restrictions on medications. **Prohibited:** The following medications are prohibited between Screening and Day 56 and administration will be considered a protocol violation. • Investigational therapies (other than the protocol defined therapy) for 28 days prior to screening visit and through Visit 3 (Day 56). • Acute antibiotics for pulmonary symptoms between screening and through Visit 3 (Day 56) unless the subject meets the criteria for a protocol defined pulmonary exacerbation (Appendix B).
EFFICACY EVALUATIONS	We have mirrored our prior STAR-too trial efficacy measures, explained below, to which comparison is made.
PRIMARY ENDPOINT	The proportion of subjects with MRSA eradicated from respiratory tract cultures at Day 28.
SECONDARY ENDPOINTS	1. Percentage of subjects who are considered to be compliant and adherent with the eradication treatment protocol 2. Change from baseline for FEV_1 and change from baseline for weight/BMI 3. The proportion of subjects with MRSA eradicated from respiratory tract cultures at Day 56 4. Assess treatment burden and compare to reports of burden in STAR-too study 5. Proportion of subjects with adverse events 6. Occurrence of any small colony variant *S. aureus* (MRSA or MSSA) in

	persistent / recurrent isolates through to Day 180
MECHANISTIC ENDPOINTS	1. Frequency of MRSA nasal colonization in any household member of the study subject.
PLANNED INTERIM ANALYSES - SAFETY	An independent data monitoring committee (DMC) will conduct a safety review every 6 months and will receive subject enrollment updates every 3 months. This DMC is sponsored by the CFFT and composed of one physician with expertise in CF, a biostatistician and a study coordinator, with the possible addition of an ad-hoc if indicated. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	The primary endpoint, the proportion of subjects with a negative culture for MRSA at Day 28, will be descriptively summarized with corresponding 95% confidence interval. The primary analysis population will be comprised of all subjects with culture results available at baseline and Day 28 as defined by the intent-to-treat-efficacy (ITT-E) population. The primary efficacy analyses will also be performed on a per-protocol efficacy (PPE) population consisting of protocol-defined compliant subjects.
Rationale for Number of Subjects	A recently completed study of an aggressive MRSA eradication protocol suggested that 82% of subjects with new MRSA who are treated will be MRSA negative at day 28 as compared to 26% of those who receive standard of care (56% treatment difference, 95% CI: 23% to 80% . The primary aim of this study is to determine whether a streamlined eradication protocol will produce comparable microbiologic efficacy at day 28. Assuming that the streamlined treatment will be comparable at 82% , a sample size of 38 subjects would provide estimation of the proportion of subjects negative for MRSA at day 28 to be 82% (31/38) with precision quantified by a confidence interval ranging from 66% -92%. The lower bound of this confidence interval (66%) does not rule out non-inferiority to the aggressive MRSA eradication protocol and would demonstrate superiority to the STAR-too observational control; the lower bound includes a range of values considered efficacious for administration of the treatment protocol.

1 BACKGROUND

Methicillin resistant *S. aureus* (MRSA) has become one of the 3rd most prevalent chronic respiratory organism in people with CF in the U.S. In recent years the national prevalence has remained around 26% with a continued rise in incidence (1). Epidemiological studies have shown that chronic MRSA infection is associated with worse lung function compared to MSSA (2, 3). Additional data suggest that chronic but not transient MRSA infection was associated with higher mortality (4) and patients with incident MRSA see an increase in all type of therapies and in number of clinic visits within 6 months of MRSA acquisition (5). Beyond these clinical concerns, isolation precautions in the healthcare setting and the limited choice of antibiotics, especially for chronic infections, complicate care and make treatment of MRSA an urgent issue. Further the possibility of spread from people with CF to other people makes MRSA a public health issue. All these concerns have led to increasing use of anti-MRSA therapy in the CF population, however there are widely different approaches ranging from no treatment if the patient is stable to aggressive eradication attempts with oral and topical antibiotics.

1.1 Overview of Clinical Studies and current standard of care

Currently U.S. guidelines for CF do not recommend treatment of MRSA or MSSA if the patient is clinically at baseline. Several retrospective or non-controlled small studies described attempts at MRSA eradication in people with CF. A recent Cochrane review concluded that there were no randomized trials available to assess efficacy of early MRSA treatment in CF (6).

Our recently completed randomized trial comparing treatment to observation i.e. current standard of care (**St**aph **A**ureus **R**esistance – **T**reat **o**r **o**bserve) showed promising results. At Day 28, 82% (n=18/22) of participants in the Treatment- arm compared to 26% (n=5/19) in Observation-arm were MRSA negative. This led to the recommendation of the Data Safety Monitoring Board for early closure of the trial as efficacy even with the small number of completed subjects was evident.

The STAR-Too trial showed microbiologic success with a very detailed, complex regimen aimed at eradication. This therapy included combination oral antibiotics (TMP-SMX or minocycline with rifampin), oral and nasal decontamination, and skin de-colonization besides extra household cleaning. Such a multi-faceted approach is unlikely to be feasible outside the research setting and not all aspects may be indicated. That trial included only subjects ages 4-45 years and those who had no increase in respiratory symptoms.

1.2 Antibiotics for MRSA and potential eradication strategies

In the current proposal and our prior eradication trial we chose trimethoprim-sulfamethoxazole (TMP-SMX) to be used at the dose recommended for CF to achieve appropriate serum levels (7). The choice was based on safety and tolerability profile, reliable oral absorption, good penetration into lung tissue, and being approved and safe for use in young children and favorable pharmacokinetics (8) (9). Also, current rates of resistance in MRSA strains collected from patients with CF appear to be low around 2.7% (10). A potential concern of repeated use of TMP-SMX is emergence of thymidine dependent i.e. small-colony variant (SCV) *S. aureus*, a phenotypic adaptation of *S. aureus* during chronic infection. Prolonged use of TMP-SMX has been reported as a risk factor when treatment duration was 12 months or longer (11).

The STAR-Too trial used TMP-SMX without appearance of SCV, showed good tolerance, and was effective in combination with rifampin. Theoretical benefits of rifampin, derived from *in vitro* or animal data, show benefit in biofilm infections but the additive effect was highly variable between antibiotics. Most clinical trials combine rifampin with fusidic acid, an antibiotic that is not available in the U.S. and with high resistance rates when used as mono-therapy. Early MRSA infection is less likely a biofilm infection and may not require the addition of rifampin as was done in the prior trial; drug toxicity and the multiple drug-drug interactions of rifampin add to the concern of using rifampin. Importantly the interaction with ivacaftor-lumicaftor is clinically very relevant as many patients may be reluctant to interrupt ivacaftor (iva-/lumicaftor)

therapy. Therefore, in our current proposal, we chose TMP-SMX or minocycline as single oral agent in this trial.

Skin decontamination was used in the prior trial based on observations in non-CF that especially SCCMec IV MRSA (USA300) is a frequent colonizer/infecting organism of the skin. Yet only one subject had intermittent colonization of the skin in the prior trial thus indicating that this procedure is rarely indicated. Therefore we will not perform skin decontamination in the current trial.

1.3 Recurrence / chronic infection with MRSA

Many clinicians observe either failure of initial therapy despite seemingly adequate antibiotic choices or a high rate of recurrence after initial treatment. The STAR-too trial showed significant eradication at day 28 (12), however decreasing differences in success over time as shown in **Table 1**.

Table 1: Culture results by study arm over time in STARtoo

	MRSA negative Day 28	**MRSA negative Day 84**
Rx arm	22/24 (82%)	13/21 (62%)
Obs arm	5/19 (26%)	7/17 (41%)
Difference (CI)	56% (25%, 74%)	21% (47%, -10%)

The factors responsible for this re-occurrence are not understood in CF. In skin and soft tissue MRSA infection several factors have been found to contribute to higher relapse rates including re-infection from the environment e.g. household or household member (13, 14). Additionally MRSA might persist in other body sites/lung in CF after an initial therapy course. For example infection with *P. aeruginosa* is typically treated with multiple cycles and the best strategy for eradication of incident *P. aeruginosa* has been tested in several studies (15-19).

Based on the decreasing MRSA negative rate seen in our prior trial and the clinical experience in non-CF we will use a repeat treatment cycle in this study.

2 STUDY RATIONALE

Our recently completed trial comparing observation (i.e. standard of care) to an intense, multi-faceted eradication regimen has shown a significant difference in achieving MRSA negative cultures in the treatment arm. However several gaps remain prior to recommending early eradiation for all. This was a complex treatment regimen and only subjects between ages 4-45 years who were clinically stable at time of MRSA detection were enrolled; it is unclear whether a simpler, clinically more feasible protocol may achieve similar microbiologic success and if a repeated treatment course should be employed.

The questions to be addressed in this trial are 1) Will the microbiologic effect of TMP-SMX without addition of rifampin be effective to achieve MRSA negative cultures at Day 28. 2) Does one repeat cycle of this therapy enhance the efficacy for MRSA negative cultures at Day 56.

2.1 Risk / Benefit Assessment

Medications: The medications used in this trial are FDA approved for the indication and dosage to be used in patients with CF.

Trimethoprim-sulfamethoxazole (TMP-SMX) is a bacteriocidal antibiotic. Side effects, primarily rashes, are reported in 2-4% of patients taking TMP-SMX (20, 21). Sputum levels of antibiotic were

close to those in serum and dose dependent (8). TMP-SMX can be used in children as young as 3 months of age and pharmacokinetics are known in this age group (9). Current rates of resistance to this drug in MRSA strains collected from patients with CF appear to be lower (2.7%) than in the more common hospital associated MRSA strains as we have shown in the investigator's MRSA observational study (STAR-CF) (9). Pharmacokinetic studies of TMP and SMX have shown that relative to patients without CF, patients with CF have shorter elimination half-lives and greater plasma clearances as well as a smaller apparent volume of distribution for TMP (consistent with CF patients reduced mass of adipose tissue) [13]. Therefore recommendations are to use high dose TMP-SMX in people with CF.

Minocycline, to be used in case of patient intolerance, has good sputum penetration reaching 50-70% of peak serum concentrations with oral administration (22). Side-effects are similar to those with TMP-SMX, and there is also low anti-microbial resistance in CF based on our data.

Study design: We will use a novel study design by using the subjects from the STAR-too trial as an external comparator group. Our external comparator group was recruited from the same sites as the current proposal. In addition, our enrollment criteria are essentially the same as in the prior study and the study design (study visits and endpoints) and study procedures are the same. Further, the subjects in this comparator group were part of a strictly protocolized randomized trial; our proposed treatment subjects will also be part of a strictly protocolized treatment regimen.

If a comparator group were included in the current trial with the aim of seeing non-inferiority, a total of 370 subjects would be required. Such a trial, while analytically rigorous, would delay the answer to an important clinical question (how to treat incident MRSA) by many years, thus essentially having a negative impact on the care of CF.

The study as proposed here will ask if eradication rate is comparable to our prior controlled intervention (81% eradication) without the use of rifampin and without the use of skin disinfection. If the results show that this is the case, it would make the treatment easier and safer for patients. It allows physicians to know to treat subjects who are on CFTR modulators/correctors with an MRSA eradication regimen without significant drug interactions. If the study demonstrates a clinically relevant lower eradication rate (i.e. 30%, the rate of spontaneous clearance or even 50%), clinicians could use this information to support continued use of a high intensity eradication protocol. Finally, addition of a new observational study cohort is ethically no longer feasible as our prior trial showed benefit of early therapy.

3 STUDY OBJECTIVES

3.1 Primary Objective

To evaluate the microbiologic efficacy and safety of a two week oral and nasal MRSA eradication treatment protocol in patients with incident MRSA without exacerbation compared to our prior trial (STAR-too). This trial will use an external comparator group from our prior trial to assess response rate and thus will address the challenges of efficiently enrolling into CF clinical trials given the current high volume of therapeutic trials.

3.2 Secondary Objectives

The secondary objectives of this study are to:

- Evaluate adherence to the MRSA eradication treatment protocol

- Assess change in clinical outcomes (FEV_1 and Weight) at Day 28, Day 56, 3 months and 6 months.
- Assess the microbiologic efficacy of repeated treatment, in regards to achieving culture negative status in those failing the first cycle or reduction of recurrence in those subjects who re MRSA negative after the first treatment course. Although low numbers this can be compared to the initial trial where many subjects received antibiotics after Day 28.
- Assess treatment burden and side effects of this streamlined protocol compared to the prior dual-antibiotic treatment.
- Compare those participating in the study to those non-participants willing to consent to having their clinical data collected during routine clinic encounters to explore if a repeat treatment cycle enhances microbiologic efficacy at Day 56 and Day 180
- Monitor for emergence of drug resistances including thymidine dependent strains after the first or second treatment cycle
- Test the hypothesis that patients with persistent/recurrent MRSA at Day 56 are infected with the genetically identical isolate over time
- Exploratory outcome: Describe the frequency of nasal colonization of household members and test if likelihood of recurrent/persistent MRSA is higher in subjects with colonized household members.

3.3 STUDY DESIGN

3.4 Study Overview

This is an open-label, multi-center interventional trial in CF patients with new MRSA isolated from the respiratory tract (oropharyngeal = OP swab, sputum, or bronchoscopy) at a clinical encounter.

Forty-two subjects with new MRSA infection will be enrolled and will receive two weeks of oral trimethoprim-sulfamethoxazole (TMP-SMX) or minocycline depending on age, allergies and antibiotic resistance of prior isolate for 14 days, and nasal mupirocin for 5 days. Subjects old enough to do so will use oral disinfectant gurgle (0.12% chlorhexidine gluconate oral rinse) for 14 days. The primary endpoint will be the proportion of positive MRSA respiratory cultures at Day 28 and this will be compared to our prior STAR-Too results.

Subjects will then have a 14 day wash-out period (i.e. no TMP-SMX or minocycline from Day 14 to Day 28) and all participants will repeat the treatment protocol from Day 29 to Day 42. Repeat cultures will be done at day 56 ± 7 days, most likely combined with their next clinic visit. Results of Day 56 cultures will be an exploratory, secondary outcome.

Subsequent visits will be 3 and 6 months later with their routine clinic appointments. Any interim clinic visits will be used to obtain repeat cultures and clinical data. Due to COVID-19 if the site does not have clinic access for the subject or coming to the CTRC or healthcare facility is considered too great a risk then remote visits will be completed for visit 3 and visit 4.

Assessment of MRSA culture status will be by OP swab for all subjects, with additional sputum in those who expectorate.

Total duration of an individual subject's participation will be six months. Total duration of the study is expected to be 54 months, which includes data analyses and publication.

<u>Remote Visits:</u>

Due to COVID-19 changes for patients who have completed visit 2, and have completed study medication i.e. those for visit 3 or visit 4.

Patients may complete remote visits for visit 3 and visit 4 if the site does not have clinic access for the subject or coming to the CTRC or healthcare facility poses a risk (infection with COVID 19). This remote visit will involve:

1.Call the family 1-2 weeks prior to the planned visit to discuss the remote visit.

2. Mail swabs for an OP-swab on the study subject and for household contacts nasal swabs. We will pre-label the swabs with the study IDs so the Core Microbiology lab can process but attach a separate label for the family in plain text e.g. "OP CF" "Mom nose", "brother nose" etc. This way they will know which one is which. We will include mailing supplies for sending those back directly to the Core Microbiology lab.

3) The day of study visit we plan to call the family and review the questionnaires with them and fill all of the history and CF Quality of Life (CFQL) questionnaires with them over the phone. As well as update case report forms.

3a) Review technique of OP swab with them and ask them to do on their child or themselves if they are adolescents or adults. The visit can also be conducted by Zoom which is considered HIPAA compliant.

Contemporaneous Clinical Care Comparison Arm: Subjects with incident MRSA who are not interested in a protocolized study but otherwise eligible will be offered an opportunity to be part of the clinical care arm. If consenting, clinical data (MRSA culture status, antibiotic treatments, lung function etc.) will be collected for exploratory analyses comparing clinical outcomes of MRSA management outside the confines of a protocolized trial.

4 CRITERIA FOR EVALUATION

4.1 Primary Efficacy Endpoint

To evaluate the microbiologic efficacy and safety of a streamlined two week MRSA eradication treatment protocol.
The comparison group will be the observational group from our prior STAR-Too trial. Additionally, the rate of MRSA negative status will be compared for subjects in the treatment group i.e. the protocol with rifampin (STAR-too) compared to without rifampin (STAR-ter).
The Day 28 time point matches the prior study end-point. The Day 28 had been selected based on the following rationale: 1) to ensure that residual antibiotics in secretions would be eliminated, thereby limiting the potential for false negative cultures; 2) to give a short window of time to allow for the possibility of re-infection from persistent environmental sources; and 3) to be able to employ our external comparator group. Longer duration of culture negativity will be assessed at month 3 and month 6 as secondary measures.

4.2 Secondary Efficacy Endpoints

Clinical Efficacy Endpoints

- Proportion of subjects with a protocol-defined pulmonary exacerbation (Appendix 2) between baseline and Day 28 requiring treatment with any oral, inhaled, or IV antibiotics (regardless of potential activity against MRSA)
- Proportion of subjects with a protocol-defined pulmonary exacerbation (Appendix 2) between baseline and Day 56 requiring treatment with any oral, inhaled, or IV antibiotics (regardless of potential activity against MRSA)
- Time to protocol-defined pulmonary exacerbation over the 6 month study
- Number of protocol-defined pulmonary exacerbations over the 6 month study
- Proportion of subjects treated with oral, inhaled, and IV antibiotics over the 6 months study and number of days of use
- Change from baseline in the respiratory domain of the CF Quality of Life Questionnaire (CFQ-R) over the 6 month study
- Change from baseline in the Cystic Fibrosis Respiratory Symptom Diary (CFRSD-CRISS) over the 6 month study
- In subjects able to reliable perform spirometry, change from baseline in FEV_1 (liters and percent predicted) over the 6 month study
- Change from baseline in weight (kg and percentile) over the 6 month study

Clinical Microbiologic Endpoints

- Proportion of subjects who are negative for MRSA at Day 28 and remain negative over the remainder of the 6 month study
- Proportion of subjects who are negative for MRSA at Day 56
- Proportion of subjects who are negative for MRSA at 3 months and 6 months
- Proportion of subjects with initial MRSA negative status at Day 28 becoming MRSA positive at Day 56, 3 or 6 months
- Proportion of subjects with MRSA that is resistant to TMP/SMX, minocycline, or mupirocin at baseline and over the 6 month study period
- Number of MRSA isolates showing genetic changes consistent with thymidine dependence (=small colony variant) at any time of the study course
- Proportion of subjects with isolation of *P. aeruginosa* (new or persistent), over the 6 month study
- Proportion of subjects with positive surveillance cultures for MRSA at nares over the 6 month study.
- Proportion of subjects who do not have a MRSA positive culture at time of the screening visit ("intermittent positive") but only positive clinical isolate.
- Proportion of subjects with MRSA isolated at the time of increased pulmonary symptoms.
- Proportion of subjects with a family member being colonized with MRSA at the nares
- Proportion of subjects/families with MRSA colonization with matching genetic MRSA strain

Adherence Endpoints

- The proportion of subjects considered being compliant and adherent with the eradication treatment protocol will be evaluated based on self-report and returned medications collected at Day 28 and Day 56.

4.3 Safety Evaluations

- Assessment of adverse events through Day 28, including clinically significant changes from screening/baseline in physical examination findings, pulse oximetry results, pulmonary function tests, and vital signs

- Microbiological safety as above

4.4 Other Exploratory Evaluations

- Risk factor analyses for non-eradication may include:
  - assessment of the impact of positive MRSA cultures from nares
  - presence of *P. aeruginosa* or specific type of MRSA (e.g. SCC*mec* typing)
  - assessment of colonization of family members or other environmental risks factors (e.g. number of children in the home, participation in sports teams etc.)
- SCC*mec* typing and PVL status of MRSA isolated at baseline will be performed for epidemiologic characterization
- Assessment of initial and, if applicable, MRSA isolates that are thymidine dependent (SCV)
- Additional molecular testing will be performed on recurrent MRSA isolates

5 SUBJECT SELECTION

5.1 Study Population

Subjects with a diagnosis of CF who meet all of the inclusion and none of the exclusion criteria will be eligible for participation in this study.

5.2 Inclusion Criteria

1. Male or female ≥ 2 and ≤ 45 years of age at the Screening Visit.
2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria:
   a. sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT)
   b. two well-characterized mutations in the cystic fibrosis transmembrane conductive regulator (CFTR) gene
   c. Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproteronol of less than -5 mV)
3. First **OR** early MRSA colonization defined as:
   a. First MRSA colonization: first documented isolation of MRSA from respiratory tract occurred ≤ 6 months prior to screening

   OR

   b. Early MRSA colonization:
      - MRSA was previously isolated from the respiratory tract ≤ 2 times over the past 3.5 years, but this was followed by at least 1 year of documented negative cultures for MRSA as noted below:
        a. At least 2 cultures performed at least 3 months apart to document 1 year of culture negativity. Each of these cultures should be documented to have been collected at least 1 week after end of any antibiotic prescription with MRSA activity.

- Patient again recently positive for MRSA from the respiratory tract (within 6 months prior to screening)

4. MRSA is available to the central laboratory – either the incident MRSA isolate from the clinic visit or the subject is MRSA positive at the screening visit
5. Clinically stable with no significant changes in health status within the 14 days prior to screening
6. 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

5.3 Exclusion Criteria

1. Received antibiotics with activity against MRSA within 28 days prior to screening (see Appendix A for list of antibiotics)
2. Use of an investigational agent within 28 days prior to screening
3. For subjects ≥ 6 years of age: FEV_1 at screening < 25% of predicted for age based on the Wang (males < 18 years, females < 16 years) or Hankinson (males ≥ 18 years, females ≥ 16 years) standardized equations
4. MRSA from the screening culture or the most recent clinical care visit within 6 months prior to screening resistant to TMP/SMX
5. History of intolerance to topical chlorhexidine or mupirocin
6. History of intolerance to **both** TMP/SMX and minocycline
7. < 8 years of age **and** allergic or intolerant to TMP/SMX,
8. ≥ 8 years of age **and** allergic or intolerant to TMP/SMX **and** MRSA isolate (from screening or clinical care visit)is resistant to minocycline
9. For females of child bearing potential: pregnant, breastfeeding, or unwilling to use barrier contraception through Day 42 of the study
10. Subjects with history of abnormal renal function will need screening labs showing normal function Abnormal renal function is defined as estimated creatinine clearance <50 mL/min using the
    - Bedside Schwartz Equation for subjects <18 years of age, and
    - Levey GFR MDRD Equation for subjects ≥ 18 years of age.
11. Subjects with a history of abnormal liver function will need to have screening labs showing normal transaminases. Liver dysfunction is defined as ≥3x upper limit of normal (ULN), of serum aspartate transaminase (AST) or serum alanine transaminase (ALT) or abnormal synthetic function
12. History of solid organ or hematological transplantation
13. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.

5.4 Study Specific Tolerance for Inclusion/Exclusion Criteria

Subjects who fail to meet one or more of the inclusion criteria or who meet any of the exclusion criteria will not be enrolled in this study. Waivers of any of the above study entry criteria will not be granted.

5.5 Screen Fail Criteria

All screen failures must be documented with the reason for the screen failure adequately stated. If a subject screen fails, they can be rescreened up to 2 times if the site staff feels they meet eligibility criteria and following confirmation from the study monitor.

5.6. Contemporaneous Clinical Care Comparison Arm

Subjects with incident MRSA who are not interested in a protocolized study but otherwise eligible will be offered an opportunity to be part of the clinical care arm. If consenting, clinical data (MRSA culture status, antibiotic treatments, lung function etc.) will be collected for exploratory analyses comparing clinical outcomes of MRSA management outside the confines of a protocolized trial. Data will be collected using a shortened Case Report Form for any clinic visits between incident MRSA and 6 months. The dates and intervals of these visits are solely determined by clinical care as there are no interventional aspects to this group.

6 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications between screening and Day 56, as medically feasible, with no introduction of new chronic therapies.

6.1 Allowed Medications and Treatments

Standard therapy for CF is allowed except for treatments noted in the prohibited medications section below. Ongoing chronic treatment (>21 days prior to screening) with Pulmozyme®, Cayston®, TOBI®, nebulized colistin, high dose ibuprofen, hypertonic saline, azithromycin, systemic steroids, or inhaled steroids, short and long bronchodilators and airway clearance is allowed. Patients will be counseled regarding drug interactions by the research coordinator and/or study pharmacist (e.g. need for barrier contraception if using oral contraceptives). CFTR specific medications (Ivacaftor, Ivacaftor/lumicaftor, or FDA approved CFTR modulator) are allowed if those have been ongoing for 3 months prior. A stable therapeutic regimen between screening and Day 28 is the goal, ideally no changes should be made up to Day 56. Unless otherwise medically indicated, subjects not using these therapies should not be started on them after screening or at any time prior to Visit 2 (Day 28) and subjects that have been using them chronically should be encouraged to continue them through Visit 2 (Day 28) (as per their scheduled dosing).

After Visit 3 (Day 56) there are no restrictions on medications.

6.2 Prohibited Medications and Treatments

The following medications are prohibited between Screening and Day 28 and administration will be considered a protocol violation.

- Investigational therapies (other than the protocol defined therapy) for 28 days prior to Screening and through Visit 3 (Day 56).
- Acute antibiotics for pulmonary symptoms between screening and through Visit 2 (Day 28) unless the subject meets the criteria for a protocol defined pulmonary exacerbation (Appendix 2)

7 STUDY TREATMENTS

7.1 Enrollment

Forty-two (42) subjects with new MRSA infection will be enrolled.

7.2 Description of the Agents Used

Subjects will be requested to use several prescription and non-prescription agents as part of the eradication protocol.

All of the prescription agents (TMP-SMX, minocycline, mupirocin and 0.12% chlorhexidine gluconate oral rinse) are commercially available generic products. After screening and prior to Day 1 the investigator at that site will determine the appropriate prescription agents and dose depending on the subject's age, weight, ability to comply with the oral rinse, ability to tolerate TMP-SMX and susceptibility of the screening MRSA isolate. Each investigative site pharmacist will dispense these agents in accordance with the investigator's written order (See Dose and Dosing Regimen below for more information).

In addition, a non-prescription agent (surface disinfecting wipes) will be used by subjects.

7.3 Supply of Study Drug at the Site

Each investigative site will provide the prescription medications for subjects. The site pharmacist will ensure that the prescription medications are charged to the study account at that site and not to the subject or the subject's insurance. The UNC will ship the surface disinfecting wipes to the investigational sites. The initial shipment will occur after site activation (i.e., all required regulatory documentation has been received by the Sponsor and a contract has been executed). Subsequent shipments will be made after site request for resupply.

7.4 Dosage/Dosage Regimen

7.4.1 PRESCRIPTION MEDICATIONS

Oral Antibiotics

- **Trimethoprim/Sulfamethoxazole** (TMP/SMX) (***SUBJECTS WHO TOLERATE TMP/SMX and whose MRSA is SUSCEPTIBLE should be prescribed TMP/SMX)***:
    a. Adult dose: 320 mg/1600 mg (2 double strength tablets) orally **twice daily for 14 days**
    b. Pediatric Dose; < 40kg: 8 mg/kg trimethoprim / 40 mg/kg sulfamethoxazole) given **twice daily for 14 days.** Dose of trimethoprim may be rounded to a maximum of 10 mg/kg/dose for ease of administration; ≥ 40 kg: use adult dose **(maximum dose is 320mg/1600 mg twice daily)**

- **Minocycline** (***ONLY SUBJECTS ≥ 8 YEARS OF AGE, WHO ARE NOT ABLE TO TOLERATE TMP/SMX should be prescribed minocycline.***)
    a. Adult dose: 100 mg orally **twice daily for 14 days**
    b. Pediatric dose, < 50 kg : 2 mg/kg orally **twice daily for 14 days** not to exceed 200 mg per day, ≥ 50 kg: refer to adult dose **(maximum dose is 100 mg twice daily)**

Topical Antibiotics

- Mupirocin 2% nasal ointment generously applied to each nostril using a cotton swab **twice daily for first 5 days**
- For subjects able to gurgle without swallowing: 0.12% chlorhexidine gluconate oral rinse **twice daily for 14 days**

7.4.2 NON-PRESCRIPTION AGENTS

Environmental Decontamination

- Wipe down high touch surfaces and medical equipment (e.g. VEST) with surface disinfecting wipes (provided) **daily for the first 21 days**.
- Wash all linens and towels in hot water once **weekly for three weeks**.

7.5 Dispensing

Prescribed medications will be dispensed by the investigative site pharmacist in accordance with the protocol. Both the prescription medications and the non-prescription agents will then be supplied by the study staff to subjects in quantities adequate for each two-week antibiotic and 3 week decontamination period. Prescription medications will be dispensed and labeled in accordance with local state Board of Pharmacy regulations.

7.6 Storage

The non-prescription study agents should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). The prescription agents should be stored by the site pharmacy in compliance with the local state Board of Pharmacy regulations for prescription drugs.

7.7 Study Drug Accountability

An accurate and current accounting of the dispensing and return of both prescription medications and non-prescription agents for each subject will be maintained on an ongoing basis by a member of the study site staff. The amount and type of each prescription medication dispensed and returned by the subject will be recorded on the study drug dispensing record.

7.8 Measures of Treatment Compliance

Subjects will be asked to complete a daily treatment log noting whether or not they have taken (or performed) each study treatment for Day 1-4 and Day 29-42. They will be asked to bring their treatment log and all study treatment containers (and any unused agents) at Visits 2 (Day 28), Visit and 3 (Day 56).

Treatment compliance will be based on the number of oral antibiotic doses reported as taken by the subject. Unused returned supplies (pill count or volume of oral suspension) will be evaluated to better assess the reliability of the subject report.

8 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 conduct of 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.

8.1 Clinical Assessments

8.1.1 Concomitant Medications

All concomitant medication and concurrent therapies from 30 days prior to screening will be documented at Screening and at all subsequent study visits. Dose, route, unit, frequency of administration, indication for administration and dates of medication will be captured.

8.1.2 Demographics

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

8.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening.

8.1.4 *P. aeruginosa* and MRSA History

A two-year history of respiratory culture results for both *P. aeruginosa* and MRSA will be recorded at Screening.

8.1.5 Recording of Clinical Respiratory Culture Results

Information regarding the microbiology results from cultures that are collected as part of the subject's clinical care will be extracted from the clinical record and entered into the study database for the closest culture result prior to the screening visit and from any cultures that may be collected at the time of a possible pulmonary exacerbation throughout the entire 6 month duration of the study.

Information 8.1.1. through 8.1.6 (with exception of DOB) will be collected on paper CRF for subjects who had one positive MRSA isolate but do not fulfill other inclusion criteria (including age range < 2 years or > 45 years). This information will be provided to the Data Coordinating center unidentified to allow additional epidemiologic study of new MRSA onset.

8.1.6 Physical Examination

A complete physical examination will be performed and recorded by either the investigator or a physician sub-investigator at Screening/Visit 1 and at Visit 2 (Day 28). Qualified staff (MD, NP, RN, PA) may complete the abbreviated physical exam at all other visits. After screening, new clinically significant abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit. At remote visits physical exam will be conducted as feasible via remote visit.

8.1.7 Weight and Height

Weight will be measured on the same scale and recorded at all visits. Adults and children may remain in clothes (without shoes). A standing height will be measured and recorded at each visit. For remote visit 3 and visit 4 we will not collect height and weight, unless patients have access to a scale at home to collect an up to date measurement.

8.1.8 Vital Signs

Resting (minimum of 5 minutes) measurements of body temperature, blood pressure, pulse and respirations will be performed and recorded at all study visits. For remote visit 3 and visit 4 we will not collect vital signs.

8.1.9 Oximetry

A resting (minimum of 5 minutes) measurement of oximetry will be measured on room air and recorded at all study visits. For remote visit 3 and visit 4 we will not collect oximetry.

8.1.10 Assessment of ability to perform oral rinse

In subject's > 3 and <10 years of age, their ability to "gurgle, but not swallow" will be confirmed using 1 tablespoon (15 mL) of water. Subjects who are not able to swish or gurgle without swallowing will not be prescribed the 0.12% chlorhexidine oral rinse.

8.1.11 Spirometry

In subjects ≥ 6 years old, spirometry will be performed in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests at all study visits. Patients who routinely use bronchodilators should use them prior to spirometry as noted below:

- Patients who routinely use short acting inhaled bronchodilators should use them 15 minutes to 2 hours prior to spirometry during study visits.
- Patients who routinely use long acting bronchodilator agents should use them 15 minutes to 6 hours prior to spirometry during study visits.

For remote visit 3 and visit 4 will not collect spirometry.

For patients enrolled after April 2020 and while COVID-19 precautions need to be in place that make research spirometry unfeasible, it will be acceptable to use spirometry results from the most recent clinic visit that is +/- 2 weeks of the study visit and the patient does not report changes in clinical status. This applies to all study visits that is not combined with a clinic visit.

If outside this window but spirometry cannot be obtained at the study visit or if the patient has had a change in clinical status prior to the visit, it will be reported as a protocol deviation.

8.1.12 Pulmonary Symptom Review

Key pulmonary signs and symptoms will be reviewed and documented at Visits 2, 3, and 4, and at any acute illness encounters that occur between screening visit and Visit 4 (Month 6).

8.1.13 Subject Questionnaires

Cystic Fibrosis Respiratory Symptom Diary (CFRSD) Chronic Respiratory Infection Symptom Score (CRISS)

The CFRSD-CRISS includes 16 questions (8 symptom items, 4 emotional impact items, and 4 activity impact items) for patient reporting. For subjects ≤ 11 years of age, the parent/guardian will complete the questionnaire, for subjects ≥ 12 years of age, the subject will complete the questionnaire. Although the instrument is designed for daily recall, in this study it will only be administered at each study visit.

CFQ-R

The CFQ-R is a patient or parent-reported quality of life instrument, regarding symptoms and mood over the preceding 2 weeks. The CFQ-R takes approximately 10 minutes to complete. The age appropriate version of the CFQ-R will be completed at each study visit by the subject and/or their parent/guardian.

Global Assessment of Protocol Burden

At Visit 2 and 3 (or at early withdrawal if withdrawal occurs prior to Visit 3) subjects will be asked to respond on a scale of 0 (very easy, no impact on my daily activities) to 10 (very inconvenient, took a lot of time) to the question: "Overall, how much of a time burden on you was participating in this study?". This information will help guide us in future research. For subjects < 16 years of age, the parent/guardian will complete the assessment, for subjects ≥ 16 years of age, the subject will complete the assessment.

8.1.14 Adverse Events

Information regarding occurrence of adverse events will be captured from screening visit through Visit 3 (Day 56) of the study. Duration (start and stop dates), severity, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

8.2 Clinical Laboratory Measurements

8.2.1 Renal and Liver function tests

Only subjects who have a history of abnormal renal or liver function will be required to have serum chemistries. These include BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, gamma-glutamyl transferase (GGT) and total bilirubin at Screening. If these are elevated but≤ 3x ULN repeat measures are required on Day 28 and Day 56. Patients who need renal and/or liver function for visit 3 test will be unable to complete remote visit 3 and will need complete these visits in person.

8.2.2 Pregnancy Test

Urine or blood (approximately 2 cc) will be collected from females who are of childbearing age for a pregnancy testing at Screening. Only for subjects who sign consent for study participation and are females who are of childbearing age: a follow-up pregnancy test will be performed at Visit 2 (Day 28).

8.3 Research Laboratory Measurements

MRSA subculture from clinic visit MRSA:

Research sites will need to obtain the MRSA isolate that grew from the clinical visit showing the first time MRSA. A subculture is obtained by picking 10 different colonies of this MRSA culture (at the local laboratory). This is done by swiping a sterile Remel Bacti-Swab from different sections of the culture plate on the day of shipment. This Remel-Bacti swab should be labeled with permanent marker indicating "MRSA clinical isolate" and site and patient study-ID. This swab with MRSA subculture is sent to UNC for MRSA confirmation and antibiotic susceptibility testing.

8.3.1 Study Cultures for MRSA and *P. aeruginosa*

At all visits the following samples will be collected:

- Oropharyngeal (OP) swab: Specimens will be collected with a sterile rayon-tipped culture swab (with transport medium) from the posterior oropharyngeal wall and tonsillar pillars. Subjects will be encouraged to cough prior to collection of the OP specimen. Sputum (if the subject is able to produce sputum) will be collected in a sterile specimen cup.
- Nares (Left/Right): A single sterile rayon-tipped culture swab (with transport medium) will be used to sample both nares.
- After visit 1 and 2 nasal swabs for MRSA will be collected from all permanent household members. This can be done at the time of the research visit for the household member at the visit and will be done by an adult (either study subject if an adult or parent for minors) after the visit. These swabs will be mailed to the study site at room temperature.
- For remote visit 3 and remote visit 4 OP, Nares swabs will be mailed to participants a week prior to their visit. Subjects will also receive instructions for completing swabs prior to the visit. On the day of visit study personnel will walk an adult member of the household through completing the swab for the study subject and any applicable household members. For subjects who produce sputum they will receive a sterile specimen cup to collect and ship samples in. These swabs will be mailed to the study site at room temperature using category B infectious material approved shipping supplies.

All specimens will be labeled to identify patient culture, household swab or MRSA isolate and sent for processing at the central microbiology lab at the McLendon Clinical Microbiology-Immunology Laboratories at the University of North Carolina Hospitals (UNC).

Shipping will occur at room temperature for all cultures. Timing: Cultures from the screening visit will be shipped by via overnight mail (Sat. delivery ok); specimens from other visits will be shipped by via FedEx 2 day shipping. The MRSA isolate from the sub-culture clinic culture can be sent at the same time as the patient samples collected at screening. Nasal swabs obtained from household members can be stored at room temperature until the next shipment to UNC. These swabs from

household members should be sent by the site study coordinator to UNC jointly with the other samples.

Patients' respiratory specimens will be cultured for presence or absence of MRSA and *P. aeruginosa*. Nares will be cultured for MRSA only.

Determination of *P. aeruginosa* status and MRSA Susceptibility testing:

The clinical visit culture results will be used for determination of absence or presence of MRSA and *P. aeruginosa.* This presence of *P. aeruginosa* in the local clinical care laboratory will be used for characterizing the subject as *P. aeruginosa* positive or negative. The subcultured MRSA isolate obtained from this clinical care culture or the MRSA isolates from screening will be used for antibiotic susceptibilities at the Core lab at UNC. These include susceptibility to TMP/SMX, and minocycline by disk diffusion technique and for mupirocin susceptibility by Etest®. Interpretation of susceptibility data will be based on 2010 CLSI criteria, including high-level resistance to mupirocin.

In case the local laboratory where the patient's clinical care culture was obtained is not a CFFT accredited site following CFF Respiratory Culture processing guidelines, or if the clinical culture is no longer available, the respiratory culture obtained at the screening visit will be used to test MRSA susceptibility and to determine *P. aeruginosa* status.

Additional molecular analyses of respiratory, nares, and household member isolates such as SCC*mec* typing and PVL status will be conducted on any MRSA isolated from baseline for epidemiologic characterization. In case of persistence or recurrence of MRSA, and the isolate being of the same *spa*-type, MRSA whole genome sequencing will be performed. This will include isolates from family members in children with persistent/recurrent MRSA.

Investigators will NOT be notified of any subsequent culture or susceptibility results from the research specimens to avoid potential bias in clinical treatment.

9 EVALUATIONS BY VISIT

9.1 Visit 1 – Screening (Day – 7 to - 10)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Review the "Consent for Collection and Use of your CFF Registry ID Number" and ensure that the subject (subject's legal representative) has checked one of the two options (Subjects who elect not to provide this information are still eligible for this study).
3. Assign the subject a unique screening/study number.
4. Record demographics data.
5. Administer the CFQ-R and CFRSD-CRISS.
6. Record medical history, including a history of CF, diagnosis date, and prior CF treatments.
7. Record the *P. aeruginosa* and MRSA culture results for any respiratory cultures collected over the last 24 months.

8. Record the complete microbiology results for the closest respiratory culture prior to the screening visit.
9. Record concomitant medications.
10. Measure and record height and weight.
11. Obtain and record vital signs.
12. Obtain and record oximetry.
13. Perform a complete physical examination by physician.
14. Perform and record spirometry (if subject is ≥ 6 years old) and if feasible in setting of COVID-19 otherwise refer to section 8.1.12
15. If indicated (history of abnormal renal or hepatic function/transaminitis), collect blood for clinical laboratory tests (chemistry).
16. Collect blood or urine for pregnancy test (if female of child-bearing potential).
17. Collect study cultures to be processed for MRSA and *P. aeruginosa*
18. Obtain the MRSA isolate from the clinical visit that lead to identification of new MRSA. This culture needs to be no longer than 6 months prior to screening,
19. Assess the patient's ability to perform oral rinse using water.

Tentatively schedule the patient to return for V2 (35-42 days after screening (based on estimated availability of results required for eligibility confirmation). Phone call confirming eligibility (7 to 10 days after screening)

Note: Day 1 is designated by start of treatment and is designated as 7- 10 days after screening. Subjects should be called for dispensing study medications as soon as results needed for eligibility confirmation are available (5-14 days).If geographic distance makes returning to pick up study medications unfeasible, the study medications can be sent via overnight mail.

1. After receiving the microbiology results from the UNC central lab (5-7 days for susceptibility testing only (i.e. MRSA isolate available locally); up to 14 days for both respiratory culture and susceptibility results after the screening visit), review all eligibility criteria to confirm eligibility.
2. For all eligible subjects: complete the appropriate data entry in paper case report form.
3. Contact all screened individuals by phone: to notify them regarding their eligibility for the study. If not eligible, ask them if it is ok to follow their clinical course through their medical chart and to collect any potential further MRSA isolates from their clinical visits.
4. During phone contact:
   - remind them to return to pick up their study medications as previously scheduled.
   - review any changes to concomitant medications and changes to baseline symptoms.
   - schedule all subjects for the Day 7 call and schedule (if not already scheduled) their Visit 2 (Day 28).
5. For treatment:

- Calculate the appropriate medications per protocol based on age, weight, toleration of TMP/SMX, ability to do oral rinse and MRSA susceptibility and provide this information to the site study pharmacist.
- Ensure that all appropriate prescription medications and non-prescription agents are placed together and complete any needed fields on the treatment log to facilitate compliance with the treatment regimen.
- Review the treatment instructions with the subject.
- In general, the subject should be instructed to begin treatment the following morning to ensure that both the morning and evening doses are taken.

9.2 Day 7 ± 2 Days (Phone call)

1. Record any adverse events and changes to concomitant medications.
2. Review adherence to study treatment; problem solve as needed to maintain adherence.
3. Remind the subject to record treatments in the treatment log.
4. Remind subjects to retain empty containers and any unused medications and bring them (along with the treatment log) to Visit 2.
5. If applicable, record prescribed changes in study medications (Section 11.1).

9.3 Visit 2 (Day 28 +2 days)

1. Administer the CFQ-R and CFRSD-CRISS.
2. Record any adverse events.
3. Review treatment log and collect and count returned study drug. Give treatment log and surface disinfecting wipes back to the subject for the last week of environmental decontamination.
4. Record changes to concomitant medications.
5. Administer the Global Assessment of Protocol Burden
6. If applicable, record prescribed changes in study medications (Section 11.1).
7. Measure and record height and weight.
8. Obtain and record vital signs.
9. Obtain and record oximetry.
10. Perform complete physical examination by physician.
11. Perform pulmonary symptom review.
12. Perform and record spirometry (if subject is ≥ 6 years old). and if feasible in setting of COVID-19 otherwise refer to section 8.1.12
13. For female subjects of childbearing potential, collect blood or urine for pregnancy test.
14. Collect cultures from household members and dispense new swabs for household nasal cultures.

15. Schedule subject for Visit 3 and remind them to bring the treatment log (and any previously unreturned containers and unused medications) to Visit 3.

9.4 Visit 3 (Day 56 + 7 days)

1. Administer the CFQ-R, and CFRSD-CRISS.
2. Administer the Global Assessment of Protocol Burden.
3. Record any adverse events.
4. Review treatment log and collect and count returned study drug. Give treatment log and surface disinfecting wipes back to the subject for the last week of environmental decontamination. (for remote visits patients will receive shipping supplies to send their treatment log, and any remaining study supplies back to the site)
5. Record changes to concomitant medications.
6. Measure and record height and weight. (For remote visits no height and weight will be recorded unless the patient has access to a scale to collect a reading the day of the appointment)
7. Obtain and record vital signs. (For remote visits vitals will not be recorded unless the patient has access to a thermometer to take their temperature to collect a reading the day of their study visit)
8. Obtain and record oximetry. (For remote visits no oximetry will be recorded)
9. Perform abbreviated physical examination. (For remote visits physical exam as feasible remotely will be conducted)
10. Perform pulmonary symptom review.
11. Perform and record spirometry (if subject is ≥ 6 years old). and if feasible in setting of COVID-19 ,otherwise refer to section 8.1.12 (For remote visits no spirometry will be performed. If the subject has clinical spirometry results from +/- 2 weeks days of their study visit those will be collected for study data.)
12. Collect study cultures for MRSA and *P. aeruginosa.* Schedule subject for Visit 4; remind them to contact the study steam if respiratory symptoms occur or increase from baseline or if hospitalized.

9.5 Visit 4 – Month 6 (Day 168 ± 7 days)

1. Administer the CFQ-R and CFRSD-CRISS.
2. Ensure that any SAEs have been reported.
3. Record changes to concomitant medications.
4. Measure and record height and weight. (For remote visits no height and weight will be recorded unless the patient has access to a scale to collect a reading the day of the appointment)

5. Obtain and record vital signs. (For remote visits vitals will not be recorded unless the patient has access to a thermometer to take their temperature to collect a reading the day of their study visit)
6. Obtain and record oximetry. (For remote visits no oximetry will be recorded)
13. Perform abbreviated physical examination. (For remote visits physical exam as feasible remotely will be conducted)
7. Perform pulmonary symptom review.
8. Perform and record spirometry (if subject is ≥ 6 years old). and if feasible in setting of COVID-19, otherwise refer to section 8.1.2 (For remote visits no spirometry will be performed. If the subject has clinical spirometry results from +/- 2 weeks days of their study visit those will be collected for study data.)
9. Collect study cultures for MRSA and *P. aeruginosa.* Schedule subject for Visit 4; remind them to contact the study steam if respiratory symptoms occur or increase from baseline or if hospitalized.
10. Complete the study termination CRF.

9.6 Acute Illness Encounter

If a subject is seen in the clinic or phones either the investigator or a clinical physician with increased pulmonary symptoms, extract the following information from the patient's medical record.

1. Record findings as available on the pulmonary symptom review case report form.
2. Record changes to concomitant medications.
3. Record the clinical microbiology results if a respiratory culture was performed.

9.7 Early Withdrawal from Study

1. If the subject is willing to come in for a follow-up visit, follow the procedures for the next scheduled visit.
2. Complete the global assessment of protocol burden if withdrawal occurs prior to Visit 3.
3. Complete the study termination CRF.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 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 study drug, whether or not related to that study drug. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Prescribing or Product Information or of greater severity or frequency than expected based on the information in the Prescribing or Product Information.

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. Adverse events will be described by duration (start and stop dates and times), severity, seriousness, outcome, treatment and relation to study drug, or if unrelated, the cause.

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 by the investigator. 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 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 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 by the investigator using the guidelines shown in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
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.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE 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.

10.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) on an SAE Report Form. 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. **NOTE: *SAEs will be collected through the end of the study*** (unlike AEs which will only be collected through Visit 3).

All SAE Report Forms will be reviewed by the site investigator and sent to the UW within one business day of the site learning of the event. Sites will fax the SAE report to:

• SAE Fax: 206-685-8673

The site will notify the UW of additional information or follow-up to an initial SAE Report as soon as relevant information is available. Follow-up information is reported on an SAE Report Form.

The site investigator will report SAEs to the IRB, in accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB).

10.3 Medical Monitoring

Kathleen Ramos, M. D., MSci (Medical Monitor) should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: (800) 341-0961
Pager: (800) 341- 0961

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

11.1 Early Discontinuation of Study Drug

Because this protocol includes multiple treatments with known adverse event profiles the following study drug discontinuation algorithm should be followed:

- Subjects with GI symptoms (i.e. temporally related to medication and significant nausea resulting in decreased oral intake or vomiting):
    - Discontinue dosing with TMP/SMX or minocycline

- Subjects with rash suspected to be related to TMP/SMX after physician evaluation of the rash
    - If the subject is >8 years of age:
        - Change the 2nd antibiotic to minocycline to complete a total duration of antibiotic dosing of 14 days (i.e. if TMP/SMX was taken for 3 days than minocycline would be dosed for 11 days).
    - If the subject is ≤ 8 years of age:
        - Discontinue all oral antibiotics, but continue with topical antibiotics and environmental decontamination

All dose modifications should be recorded appropriately in the source documents and in the CRF.

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

- Subject withdrawal of consent (or assent)
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject discontinues all oral antibiotics due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue oral treatments early will be encouraged to complete all remaining scheduled visits and procedures.

11.2 Withdrawal of Subjects from the Study

All subjects are free to withdraw from participation in the study at any time, for any reason, specified or unspecified, and without prejudice. This may include subjects who withdraw from study treatment early but who decline to continue to come in for remaining follow-up visits or it may include subjects who completed treatment and decline to come in for remaining follow up visits. A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

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 should be encouraged to come in for a final visit (Section 9.9).

11.3 Replacement of Subjects

Subjects who withdraw from the study treatment or from the study will not be replaced.

12 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or the Sponsor and their designees fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Inappropriate dosage of study medication administered

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject from the study or study treatment.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files. The site will report the violation to their IRB in accordance with their IRB reporting requirements.

13 DATA SAFETY MONITORING

The Cystic Fibrosis Foundation Data Safety Monitoring Board (DSMB) will establish a data monitoring committee (DMC) that will consist of one physician (Wayne Morgan, M.D.), a biostatistician, a DMC study coordinator and an ad-hoc at Dr. Morgan's discretion to review data relating to safety and to review subject enrollment. Dr. Morgan will receive enrollment reports every 3 months and conduct safety reviews every 6 months 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 Data Monitoring Charter to be established for this protocol. Further details regarding the timing and content of the interim reviews is included in the statistical methods section below.

14 STATISTICAL METHODS AND CONSIDERATIONS

Prior to open enrollment across all sites, 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.

14.1 Data Sets Analyzed

Several analysis populations will be utilized for the analyses.

14.1.1 Intent- to-Treat (ITT) Population

The ITT population will be defined as all subjects who sign consent and receive study drug. All subjects will be evaluated for safety and tolerability if they are part of the ITT population.

14.1.2 Intent-to-Treat Efficacy (ITT-E) Population

Subjects in the ITT safety population who were assessed for the primary microbiologic efficacy endpoint at baseline and day 28 will be included in the ITT efficacy population that will be used for the primary efficacy analyses.

14.1.3 Per-Protocol Efficacy (PPE) Population

Subjects in the ITT-E population who have no major protocol violations during the first 28 days of the study and who are compliant with oral antibiotics (as described below) will be included in PPE population for which sensitivity analyses of the primary efficacy endpoint will be performed.

- Non-compliance in the first 28 days is defined as either: (1) <80% compliance with oral rifampin as calculated by: (number of doses taken/28)*100 or for pediatric patients who receive oral suspension (number of doses taken/28)*100 (2) <80% compliance with TMS/SMX or minocycline as previously defined.

14.1.4 Observed Cases (OC) Population

Analyses of secondary efficacy endpoints will include subjects who had an assessment at follow-up visits specific to the analyses. The effective sample size at each assessment visit may differ as a result of premature patient discontinuation or failure to comply with the evaluation schedule.

14.2 Demographic and Baseline Characteristics

Subjects will be described and compared with respect to baseline demographic and clinical characteristics including but not limited to age, gender, race, genotype, height, weight, pulmonary function, and microbiology. For all analyses, baseline will be defined as the measurement taken at Visit 1 (Screening).

14.3 Analysis of Primary Endpoint

The primary endpoint, the proportion of subjects with a negative culture for MRSA at Day 28, will be descriptively summarized with corresponding 95% confidence interval. The primary analysis population will be comprised of all subjects with culture results available at baseline and Day 28 as defined by the ITT-E population. The primary efficacy analyses will also be performed on a per-

protocol efficacy (PPE) population consisting of protocol-defined compliant subjects. The proportion of subjects with a negative culture for MRSA at Day 28 will be compared to the treatment arm of the recently completed STAR-Too trial (Table 3).

This novel trial design i.e. use of recent, external comparators from a randomized trial will address the difficulty of implementing prospective controlled studies in rare patient populations and is sensitive to the current high volume of trials. This decision also takes into account that investigators no longer feel that they have equipoise to withhold a MRSA eradication protocol from their patients as was done in the STAR-Too trial.

Table 3: **STAR-Too Results noting differences between the treatment (Rx) arm and observation (Obs) arm**

	MRSA negative Day 28	**MRSA negative Day 84**	**Exacerbations to Day 28**
Rx arm (Treatment Regimen)	22/24 (82%)	13/21 (62%)	3
Obs arm (Control Regimen)	5/19 (26%)	7/17 (41%)	7
Difference (CI)	56% (25%, 74%)	21% (47%, -10%)	21% (44, -4%)†

† % Difference in study arms for those subjects having at least one pulmonary exacerbation.

To evaluate the robustness of primary study results, multiple sensitivity analyses for the primary endpoint will be conducted. In particular, the results will assess the robustness of study results by restricting the analysis to patients who do not require treatment for MRSA after Day 14 and prior to Day 28. Additional analyses will assess the impact of missing culture data at Day 28 by simulation studies which assume the missing cultures are either negative or positive.

14.4 Analyses of Secondary Endpoints

Secondary Clinical Efficacy Endpoints

- Proportion of subjects with a protocol-defined pulmonary exacerbation (Appendix 2) between baseline and Day 28 who are treated with antibiotics active against MRSA
- Proportion of subjects with a protocol-defined pulmonary exacerbation between baseline and Day 28 requiring treatment with any oral, inhaled, or IV antibiotics (regardless of potential activity against MRSA)
- Time to protocol-defined pulmonary exacerbation over the 6 month study
- Number of protocol-defined pulmonary exacerbations over the six month study
- Proportion of subjects treated with oral, inhaled, and IV antibiotics over the 6 month study and number of days of use
- Change from baseline in the respiratory domain of the CFQ-R over the 6 month study
- Change from baseline in the CFRSD-CRISS over the 6 month study
- In subjects ≥ 6 years of age, change from baseline in FEV_1 (liters and percent predicted) over the 6 month study
- Change from baseline in weight (kg and percentile) over the 6 month study

The proportion of subjects with a protocol-defined exacerbation will be descriptively summarized by study arm with corresponding 95% confidence intervals and tested using a two-sided 0.05 level chi square test. Time to first exacerbation over the 6 month study will be graphically displayed for each treatment group using Kaplan-Meir estimates, and a corresponding hazard ratio will be estimated using Cox proportional hazards regression. Number of pulmonary exacerbations will be summarized by treatment group and comparisons in rates performed using Poisson regression or non-parametric testing procedures as appropriate.

Descriptive statistics will be used to summarize the proportion of subjects initiating acute antibiotics (by route) and differences in proportions between the study arms will be estimated with accompanying 95% confidence intervals. Exploratory testing using point and interval estimates of the proportions and differences between proportions will be performed. Descriptive analyses will be used to summarize CFQ-R respiratory domain data and CFRSD-CRISS data collected throughout the study.

Descriptive statistics and graphical displays will be used to summarize pulmonary function parameters (FEV_1 [liters] and FEV_1% predicted) both at each study visit and changes from baseline. Differences between study arms with respect to the 28-day changes will be estimated with 95% confidence intervals. Exploratory testing of the 28-day change will be performed using two-sample comparisons, which focus on point- and interval-estimates. Longitudinal linear regression models will be utilized to model changes in pulmonary function over the 6 month study and to estimate differences between study arms. Analogous methods will be utilized for analyzing weight (kg and percentile).

Clinical Microbiologic Endpoints

- Proportion of subjects who are negative for MRSA at Day 28 and remain negative over the remainder of the 6 month study
- Proportion of subjects who are negative for MRSA at 3 months and 6 months
- Proportion of subjects with MRSA that is resistant to TMP/SMX, minocycline , and mupirocin after baseline and over the 6 month study
- Proportion of subjects with new isolation of *P. aeruginosa* over the 6 month study
- Proportion of subjects with positive surveillance cultures for MRSA at other body sites (i.e. nares and skin) over the 6 month study
- Proportion of subjects who do not have a MRSA positive culture at time of the screening visit ("intermittent positive") but only positive clinical isolate.

The proportion of subjects with emergent or resistant organisms will be descriptively summarized both within the first 28 days and over the 6 month study with accompanying 95% confidence intervals.

Safety

- Incidence of adverse events

Patients who received at least one dose of study drug and who are discontinued from all oral antibiotics or who undergo dose modification as described in Section 11.1 will be descriptively summarized. All reported adverse events will be mapped to a standard classification system and grouped by body system. Adverse events will be tabulated using standard coding terms and sorted by body system. The incidence of serious and non-serious adverse events within the first 56 days of

the study will be tabulated. If an adverse event is reported more than once during the study period for a given patient, the greatest severity and the worst-case attribution will be presented in tables. Adverse events will also be listed for individual patients, along with information regarding onset, duration, severity, and relationship to study drug.

Compliance and Adherence

- Proportion of subjects with >80% compliance for study drug during the first 28 days, defined as:
    - Defined as either: >80% compliance with TMP/SMX or minocycline as previously defined.
- Proportion of subjects with adherence to topical antibiotic usage during the first 28 days, defined as:
    - 1) <2 reported days of twice-daily missed nasal ointment, (2) <2 reported days of missed body wash with 2% chlorhexidine, (3) <4 reported days of missed twice daily oral rinse with 0.12% chlorhexidine.

The proportion of subjects who are compliant or adherent will be descriptively summarized with accompanying 95% confidence intervals.

14.5 6-month Safety and Patient Enrollment Reviews

Oversight for this trial will be by a Data monitoring committee (DMC) appointed by the CFF Data Safety Monitoring Board (Wayne Morgan, M.D.). This committee will consist of one physician (Wayne Morgan, M.D.), a biostatistician, a DMC study coordinator and an ad-hoc at Dr. Morgan's discretion. All serious adverse events will be reported to the DMC on an ongoing basis throughout the study. In addition, the DMC will receive enrollment reports every 3 months and conduct safety reviews every 6 months to ensure the continued scientific validity and merit of the study. This study has no pre-specified stopping rule for futility or efficacy. All study personnel and investigators will remain blinded to results of the interim reports, with the exception of key data management and statistical personnel who will prepare the interim reports.

14.6 Choice of Sample Size

A recently completed study of an aggressive MRSA eradication protocol suggested that 82% of subjects with new MRSA who are treated will be MRSA negative at Day 28 as compared to 26% of those who receive standard of care (observation only) (56% treatment difference, 95% CI: 23% to 80%). The primary aim of this study is to determine whether a streamlined eradication protocol will produce comparable microbiologic efficacy at Day 28. Assuming that the streamlined treatment will be comparable at 80%, a sample size of 42 subjects giving us at least 38 evaluable patients would provide estimation of the proportion of subjects negative for MRSA at Day 28 to be 82% (31/38) with precision quantified by a confidence interval ranging from 66% to 92%.
If we observe an 82% rate of success (31/38), then compared to the rate in STAR-too (82% [61,93]%), the 95% C.I. for the difference would be [-22, 19]%.
We anticipate a 10% attrition rate, and thus will need to enroll 4 additional patients along with the 38 patients we expect to complete the study. Thus sample size to recruit is set at 42 subjects.

The lower bound of this confidence interval (66%) does not rule out non-inferiority to the aggressive MRSA eradication protocol and would demonstrate superiority to the STAR-too observational control; the lower bound includes a range of values to be considered efficacious for administration of the treatment protocol.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific paper Case Report Form (CRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, subject number and initials.

If a correction is required for a CRF, the time and date will need to be added to the form by the person entering or updating CRF data.

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.

15.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group at the University of Washington will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

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

15.3 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 on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, 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 (if applicable) 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 (e.g. patient files, signed informed consent forms, copies of CRFs, study essential documents, etc.) must be kept secured on site for a period of one year after database lock and accessible for 10 years following database lock. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Part 312 and ICH Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP. By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

15.7 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 Sponsor. If specific consent is given, the subject's CFF patient registry number will also be collected. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

16 ADMINISTRATIVE, ETHICAL, 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 only. 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).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments can not be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. 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.

16.2 Institutional Review Boards

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the

Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, prescribing information, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's 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. The IRB's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. 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 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 and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may adversely affect 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.

16.3 Informed Consent Form

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][i], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonization and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

During the course of the study, if modifications are made to the consent form that impact the subject, the subject will be re-consented as described above.

16.3.1 Consent for collection and use of CFF Registry ID Number

To facilitate possible future evaluation of long-term outcomes information from all patients who screen for this study, consent will also be sought to collect the subject's CFF Registry ID number at the screening visit. The CFF registry collects data on all CF patients followed at CFF-accredited care centers at each clinical encounter, at each hospitalization or course of antibiotics, and via a year-end survey. Data include microbiology results, spirometry results, CF genotype and other information. If specific consent is given to collect this number, the patient's CF registry number will be recorded in the CRF.

16.4 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 study Sponsor 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.

16.5 Investigator Responsibilities

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

14. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
15. Personally conduct or supervise the study (or investigation).
16. 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.
17. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
18. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
19. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
20. 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.
21. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
22. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
23. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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APPENDIX 1. SCHEDULE OF EVENTS

	VISIT 1 SCREENING (Day -7 to Day -10 (BASELINE)	PHONE CALL (DAY 7)[a,b]	VISIT 2 (Day 28)[b]	PHONE CALL (DAY 36) [a,b]	VISIT 3 (DAY 56)[c] Poss. Overlap w 3 monthly clinic visit	VISIT 4 (DAY 168±7) MONTH 6 (W CLINIC VISIT)	ACUTE ILLNESS CRFS
Informed Consent	X						
Consent for Registry Number	X						
Medical History	X						
MRSA Risk Factors	X						
P. aeruginosa /MRSA History	X						
Clinical Care Microbiology Culture Results[d]	X						X
Complete Physical Exam	X		X				
Abbreviated Physical Exam [m]					X	X	
Height [n]	X		X		X	X	
Weight [m]	X		X		X	X	
Vital Signs [m]	X		X		X	X	
Oximetry [n]	X		X		X	X	
Spirometry[e, m]	X		X		X	X	
Microbiology Cultures[f, m] Sputum (if possible) *OP – all patients* Nares (left and right)	X		X		X	X	
Pregnancy Test (Urine or Serum)[g]	X		X				
Liver, renal function if history of abnormalities in past (see Section 5)	X						
Assess ability to oral rinse[h]	X						
CFQ-R (Respiratory)[l]	X		X		X	X	

Dispense Study Drugs/Treatment Log	**X**		**X**				
Teach swab from household members and dispense swabs	**X**		**X**				
Collect household member swabs [m]			**X**		**X**		
Drug accountability[i,l]			**X**		**X**		
Global Assessment of Protocol Burden [l]			**X**		**X**		
CFRSD Questionnaire[j,l]	**X**		**X**		**X**	**X**	
Concomitant Medication Review [l]	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Adverse Events [l]		**X**	**X**	**X**			
Pulmonary Symptom Review[k,l]			**X**		**X**	**X**	**X**

[a] Phone call check in for concomitant medications, adverse events and reminder about treatment adherence

b +2 days

c +7 days

d Results from the most recent culture collected for clinical care prior to the screening visit will be entered into the study database to document the presence of significant CF pathogens. During the study if a culture is collected to identify potential pathogens during a pulmonary exacerbation, results will be entered into the study database.

e In subjects ≥ 6 years old

f Respiratory tract cultures will be tested for the presence/absence of both MRSA and *Pseudomonas aeruginosa*. Nares (L, R on same swab) will be tested for the presence/absence of MRSA. MRSA isolates from respiratory tract cultures will be tested for susceptibility to rifampin, TMP/SMX, minocycline and mupirocin. Investigator's will not be provided with these data during the study (except the culture results from the screening visit to document eligibility).

g Females of child-bearing potential

h In subjects < 10 years of age

i Drug accountability will include assessment of the Treatment Log and pill count (or volume assessment of oral suspensions) The Cystic Fibrosis Respiratory Symptom

j Diary (CFRSD) will be administered at all study visit.

[k] Pulmonary Symptoms will be reviewed and documented at all study visits and at any acute illness visits during the 6 month duration of the study.

l Will be conducted without modification if subject requires remote visit 3 or remote visit 4.

m Will be conducted with modifications if subject requires remote visit 3 or remote visit 4. Refer to 8.1.2 for modifications to spirometry

n Will not be conducted if subject requires remote visit 3 or remote visit 4.

APPENDIX 2. CRITERIA FOR PROTOCOL DEFINED EXACERBATION

For this protocol, the presence of a pulmonary exacerbation is established by the following:

(1) One of the major criteria alone
or
(2) Two of the minor signs/symptoms and fulfillment of symptom duration

Please note that the presence of a pulmonary exacerbation does not *mandate* therapy with antibiotics. Rather, this definition is the *minimal* criterion for treatment with antibiotics.

Major Criteria: *(One finding alone establishes the presence of a pulmonary exacerbation)*

(1) Absolute decrease in FEV_1 of ≥10% from Visit 1 (baseline), unresponsive to albuterol (in participants able to reproducibly perform spirometry)
(2) Oxygen saturation <90% on room air or absolute decrease of ≥ 5% from Visit 1
(3) New lobar infiltrate(s) or atelectasi(e)s on chest radiograph
(4) Hemoptysis (more than streaks on more than one occasion in past week)

Minor Signs/Symptoms: *(Two minor signs/symptoms are required with duration criteria in the absence of major criteria)*

(1) Increased work of breathing or respiratory rate
(2) New or increased adventitial sounds on lung exam
(3) Weight loss ≥5% of body weight or decrease across 1 major percentile in weight percentile for age in past 6 months
(4) Increased cough
(5) Decreased exercise tolerance or level of activity
(6) Increased chest congestion or change in sputum

Sign/Symptom Duration:

- Initial symptom must have occurred for at least 5 days