Protocol Title: Efficacy and Safety of Adjuvant Topical Irrigation in the Treatment of Acute Exacerbation of Chronic Rhinosinusitis Following Functional Endoscopic Sinus Surgery (FESS)

Principal Investigator:
Nithin D. Adappa, M.D.
Dept. of Otorhinolaryngology
3400 Spruce St, Ravdin 5th Floor
215-662-2360
Nithin.Adappa@uphs.upenn.edu

Regulatory Sponsor:
Bert W. O'Malley, Jr., M.D.
Dept. of Otorhinolaryngology
3400 Spruce St, Ravdin 5th floor
215-349-5383
Bert.OMalley@uphs.upenn.edu

IRB Protocol Number: 821731

IND Number: 128711

ClinicalTrials.gov Number: 02630472

Initial version:	12/04/2014
Amended:	11/12/2015
Amended:	03/01/2016
Amended:	05/24/2016
Amended:	06/30/2016
Amended:	09/27/2016
Amended:	02/08/2017
Amended:	05/11/2017
Amended:	07/11/2017

Table of Contents

List of Abbreviations

CRS	Chronic Rhinosinusitis
NO	Nitric Oxide
FESS	Functional Endoscopic Sinus Surgery
HUP	Hospital of the University of Pennsylvania
CRF	Case Report Form
AE	Adverse Event
SAE	Serious Adverse Event
IDS	Investigational Drug Service

Study Summary

Title	Efficacy and Safety of Adjuvant Topical Irrigation in the Treatment of Acute Exacerbation of Chronic Rhinosinusitis Following Functional Endoscopic Sinus Surgery (FESS)
Short Title	Topical Irrigation Therapy for CRS
Protocol Number	821731
Phase	II
Methodology	Randomized, double-blinded, placebo-controlled clinical trial.
Study Duration	3 years for accrual and follow up (+1 year for data analysis)
Study Center(s)	Single-center: Dept. of Otorhinolaryngology at HUP
Objectives	To evaluate the physiological differences between quinine-saline vs sucrose octaacetate-placebo nasal irrigations in subjects with CRS (both with and without polyps) as well as allergic fungal sinusitis.
Number of Subjects	Number of subjects projected for the entire study: 100
Diagnosis and Main Inclusion Criteria	Chronic Rhinosinusitis post FESS
Study Product, Dose, Route, Regimen	Nasal instillation of quinine sulfate and saline solution using a 3cc syringe adopted with an atomizer.
Duration of administration	Total duration of drug product administration (including any open-label lead-in, if applicable) is twice/day for 28 days.
Reference therapy	Desroisers et al. (2001) findings showed that topical quinine in saline nasal solution was comparable, if not better to topical tobramycin (a commonly used antibiotic) for treatment of bacterial sinusitis.
Statistical Methodology	Longitudinal linear mixed-effects models to evaluate pre- and post-treatment continuous outcome scores.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Sinusitis is a common disorder accounting for an estimated 13 million physician office visits in the United States each year[1]. The aggregated cost of sinusitis is approximately $8 billion annually[2], affecting an estimated 16% of the population in the United States[1]. Despite multiple attempted treatments, including an estimated 550,000 surgeries per year[3], the disease continues to be a major health problem, both in expenditures and poor quality of life. Recent analysis of data from the National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey from 2006 to 2010 showed that rhinosinusitis accounted for more outpatient antibiotic prescriptions in adults than any other diagnosis[4].

Chronic rhinosinusitis (CRS) represents a considerable subset of this population and accounts for a significant portion of expenditures and the vast majority of surgeries. It is defined as signs and symptoms of sinusitis lasting more than 12 weeks[5,6]. Unlike the organisms responsible for acute rhinosinusitis, difficult to treat bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Stenotrophomonas multiformia* are often offending pathogens in CRS[7]. Their prevalence increases in those patients who have already had sinus surgery and continue to get recurring sinus infections. *Staph aureus* and gram-negative organisms have been shown to account for roughly 60% of infections in those patients who have previously undergone endoscopic sinus surgery[7].

Due to increasing drug resistance as well as the potential for biofilm formation, there has been an increasing pressure from both patients and clinicians alike to develop alternative treatments to systemic antibiotics. One commonly used alternative in patients who have had previous sinus surgery is topical saline irrigation with and without other topical preparations. Topical irrigations have much greater paranasal sinus penetration in post surgical patients[8]. Commonly used topical preparations include: saline alone or saline mixed with mupirocin, gentamicin, tobramycin, ceftazadine, betadine, manuka honey, baby shampoo, budesonide or mometasone[9].

We have recently identified a novel arm of upper airway innate immunity mediated by bitter taste receptors[10-15]. When a subset of airway bitter taste receptors is activated they stimulate the respiratory epithelium to generate nitric oxide, an important component of sinus innate immunity that increases mucociliary clearance as well as diffuses into the mucus where it is bactericidal. A topical therapy to activate these taste receptors may help the sinuses clear infections through this natural innate defense mechanism. While we have identified multiple bitter compounds that stimulate this response, quinine piqued our interest as it activates multiple bitter taste receptors and has already been used in the human nose.

1.2 Investigational Agent

Quinine sulfate (USP Grade) as well as Sucrose Octaacetate (USP Grade, from Spectrum Pharmaceuticals, Nenderson, NV). Both are commonly used as agents to mimic a bitter taste in placebos at Penn IDS.

1.3 Preclinical Data

Using primary human sinonasal epithelial cells loaded with the intracellular dye DAF-FM we have screened multiple bitter tasting compounds[12,13]. We have found that quinine stimulates robust NO production in a dose dependent manner that is mediated via T2Rs. Sucrose Octaacetate (used to wean babies off pacifiers) does not stimulate sinonasal epithelial cells to produce NO.

Repetitive treatment (daily application) of Quinine (1mg/ml) to primary human sinonasal cultures does not demonstrate tachyphylaxis regarding NO production, nor demonstrates any cellular toxicity.

1.4 Clinical Data to Date

In a 2001 study by Desrosiers et al[16], topical tobramycin was being evaluated for efficacy in medically recalcitrant bacterial sinusitis. In order to blind the control group, quinine was added to saline to mimic the bitter taste of tobramycin. Patients were treated with nasal solutions three times per day for 4 weeks. Interestingly, both treatment arms demonstrated clinical improvement, with the saline+quinine arm demonstrating comparable if not better efficacy to topical tobramycin with no adverse events reported in the study. These findings were surprising and "unexplained" to the authors at the time. We now believe that the quinine in their "control" group stimulated airway bitter taste receptors to stimulate sinonasal nitric oxide production to eradicate the infection. We should also mention that tobramycin does not stimulate NO production in primary human sinonasal epithelial cells.

Additionally, safety of quinine administration is further supported by a prior 1993 study that utilized quinine to determine compliance with nebulized tobramycin in the management of CF pseudomonas[17]. In this study the pharmacokinetics of inhaled tobramycin was evaluated. To assess compliance with the protocol both the tobramycin and placebo arms were spiked with quinine (1mg/ml) and urine quinine concentrations were checked. No adverse events were reported for the use of quinine in the airway.

Thus, we propose to repeat the Desrosiers study, but to instead evaluate quinine as a therapeutic agent, not a placebo, and directly compare quinine to saline (spiked with Sucrose Octaacetate). Truth be told, we stumbled onto the biologic effect of quinine in the search for a bitter compound to use in a randomized trial with another bitter compound. Surprisingly, quinine stimulated a more robust NO response than our original compound of interest (phenylthiocarbamide). Subsequently, we discovered the 2001 Desrosiers study that further supported the concept of quinine as a topical therapy for bacterial sinusitis.

1.5 Dose Rationale and Risk/Benefits

Our plan is to first study quinine against saline to determine efficacy and safety. The vast majority of patients with rhinosinusitis utilize low pressure / high volume (240mls) sinonasal lavage to cleanse the sinonasal cavity[9]. We propose to have the patients administer 6mls of quinine (1mg/ml) (same concentration that was used in the study mentioned above, which also activates NO production from human primary nasal cells *in vitro*) via a 3cc syringe with a mucosal atomizing device (MAD) on the tip. Patients will apply 3 mls to each nostril twice per day. Thus the patients will be exposed to a maximum of 12mls or 12.0 mg of quinine. In standard tonic water, quinine is 8.3mg/100mls and thus an 8oz glass of Canada Dry tonic water has 19.6mg of quinine. Thus, the maximum systemic exposure in our study (assuming ingestion of the total nasal administration) is less than drinking one glass of tonic water / day. To put this in context, the therapeutic range of quinine to treat malaria is 10mg/kg TID (2100mg for a 70kg individual) nearly 200 X the dose we are proposing.

We propose to enroll only patients who have had prior functional endoscopic sinus surgery (FESS) for two reasons: (1) better access to the sinonasal cavity to obtain microbiologic samples before, during and after the trial, and (2) patients with a marsupialized sinonasal cavity are not at risk for developing intra-orbital or intra-cranial complications from bacterial sinusitis, as a path of egress for the infection is widely patent into the nose.

2 Study Objectives

The overall objective of this study is to evaluate the efficacy of quinine nasal irrigations as a first line therapy for acute exacerbations of uncomplicated chronic rhinosinusitis following endoscopic sinus surgery.

2.1 Primary Outcome Variable(s)

The primary outcomes variables are:

- Pre- and post-treatment endoscopically guided sinonasal microbiologic cultures
- Pre- and post-treatment nasal endoscopy scored with the Lund-Kennedy scoring system by a blinded investigator[18].
- Pre- and post-treatment quality of life questionnaires (22-item Sinonasal Outcomes Test [SNOT-22]).
- The necessity for rescue oral antibiotics for persistent infection.

2.2. Secondary Outcome Variable(s)

The secondary outcomes variables are:

- Pre- and post-treatment Sniffin' Stick-12 (Burghart Instruments, Wedel, Germany) score.
- Pre- and post-treatment CT scan scored with the Lund-MacKay CT scoring system [19] by a blinded investigator. The CT scans at pre and post will only be done if clinically indicated by the physician.
- Pre- and post-treatment microbiome analysis
- Pre-treatment taste test to determine bitter taste sensitivity

3 Study Design

3.1 General Design

- This is a Randomized, double-blinded, placebo-controlled protocol
- The duration of subject participation is approximately 12 weeks
    - (Week 0) Screening/Baseline:
        - Microbiome swab obtained
        - Sniffin' Stick-12
        - Nasal Endoscopy (Lund-Kennedy Score)
        - CT scan (Lund-MacKay CT Score) if clinically indicated.
        - SNOT-22 survey
        - Nasal Irrigation distributed (Week 0)

4 week period of nasal irrigation administration (2 times a day)

    - (Week 4)
        - Microbiome swab obtained
        - Nasal Endoscopy (Lund-Kennedy Score)
        - Sniffin' Stick-12Microbiology/traditional swab*
        - SNOT-22 survey
    - (Week 12) Return to clinic for assessment of durable changes:
        - Microbiome swab obtained
        - Sniffin' Stick-12
        - Microbiology/traditional swab*
        - SNOT-22 survey
        - CT scan (Lund-MacKay CT Score) if clinically indicated

* Microbiology/traditional swabs are part of routine care if pus is evident during routine clinical nasal endoscopy.

3.2 Primary Study Endpoints

- Efficacy of quinine and saline irrigations compared to saline irrigations alone.
- Changes in quality of life (SNOT-22 questionnaire) (both composite and subscale scores)
- Nasal endoscopy (Lund-Kennedy Score)
- Alterations in microbiology recovered (as determined by sinus microbiology culture)
- Whether or not conventional antibiotics were needed to treat the infection during or following the irrigation trial (as prescribed as part of routine care)

3.3 Secondary Study Endpoints

- Changes in olfaction as quantified with the Sniffin' Stick-12 score [administered as part of research]
- Pre and post treatment CT scan as scored by the Lund-MacKay CT scoring system [19]. These scores will only be available if CT scans are clinically indicated at the time of baseline and/or 12-week post clinical visits.
- Alterations in the Sinonasal microbiome (conducted as part of an ongoing research project)

3.4 Primary Safety Endpoints

- Tolerability (due to bitterness).

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Patients diagnosed with chronic rhinosinusitis, with and without polyps, as well as allergic fungal sinusitis
2. Patients who have undergone Functional Endoscopic Sinus Surgery (FESS)
3. Purulent drainage on nasal endoscopy
4. Male or female subjects, 18 years of age or older
5. Patients seen at the Dept. of Otorhinolaryngology clinic at HUP, a tertiary care clinic.

4.2 Exclusion Criteria

1. Pregnant women
2. Immunocompromised patients
3. Granulomatous diseases with rhinologic manifestations (Wegner's, Sarcoid, Churg-Strauss)
4. Primary ciliary dyskinesia

4.3 Subject Recruitment and Screening

All study personnel will be appropriately trained and certified according to their role in the study and duties, including HIPAA and CITI training.

Subjects will be identified and approached as they present to clinic for their routine treatment for chronic rhinosinusitis. Subjects will be brought into a private clinical examination room where a clinical research nurse will explain the protocol in detail. Subjects will have the time to ask and have questions answered about the research prior to signing the consent form.

Patients interested in enrolling in the study will be instructed on how to administer the solutions using a 3cc syringe with an atomizer and have the opportunity to trial this technique with isotonic buffered saline under direct supervision of the treating physician or research coordinator. Patients who feel that they can adequately self-administer the solution will be asked to participate in the study.

Subjects who withdraw or are otherwise not interested in the protocol will have access to the ENT clinic for their regular care. They will be reminded that their care (and if applicable, employment, or student status at Penn) will in no way be affected by their decision to participate in the protocol or not.

As per FDA recommendation, the first 10 patients enrolled into the study will be placed into the treatment arm, and will not be randomized. The purpose of this is to make sure that the treatment is efficacious enough to rationalize going forward with the trial. This will not affect the ICF, as the patient will not be aware of this forced assignment into the treatment arm (i.e. they will still be blinded to their assignment).

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subject may be withdrawn from the study prior to the expected completion of the protocol if the subject fails to adhere to protocol requirements, subject withdraws consent, or symptoms of acute rhinosinusitis exacerbation persist. Subjects will return to routine clinical care at that point.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Even though subjects may be prematurely withdrawn from the study, it is imperative to collect infection data (microbiology swabs) or symptomatic data. Such data is important to the integrity of the final study analysis since early withdrawal could be related to the tolerability profile or the ineffectiveness of the therapy. If a subject withdraws consent to participate in the study, attempts should be made to obtain permission to follow up with the subject's CRS for the duration of the 12-week follow-up period. Subjects with incomplete/missing data will not be included in the analysis.

5 Study Drug

5.1 Description

IDS will compound 0.9% buffered sterile NaCl for irrigation with USP grade quinine to a 1mg/ml concentration. For the placebo arm, IDS will compound 0.9% sterile NaCl for irrigation with sucrose octaacetate (0.5mg/ml) to achieve the same degree of bitter taste as 1mg/ml concentration of quinine[19].

5.2 Treatment Regimen

1 mg/ml concentration of quinine in saline solution will be given to participants to instill in each. Doses will be administered by use of an LMA® MAD NASAL™ Intranasal Mucosal Atomization Device with 3mL syringe and vial adapter (Teleflex Medical, Research Triangle Park NC; item # MAD140OS), which is patient-specific, or other comparable device. If using a different device, the Sponsor will provide a statement confirming the devices' comparability or equivalence. Participants will do this twice per day for 4 weeks.

5.3 Method for Assigning Subjects to Treatment Groups

Urn Randomization[20] will be used to assign subjects to treatment groups. Urn randomization is a type of adaptive biased-coin randomization, where the probability of being assigned to the treatment arm changes as the balance of participants in the treatment and placebo groups changes. For example, when the first patient is added, assignment is based on simple randomization. If that patient is assigned to the treatment arm, then the probability of the 2nd patient being assigned to the treatment arm is decreased by a probability *p.* As patients are recruited, the probability of being assigned to either arm adapts in response to the size of each arm at the time of recruitment.

5.4 Preparation of Study Drug

IDS will prepare and record the distribution of the irrigant. The Clinical Research Coordinator or Clinical Research Nurse will pick up the solutions and will demonstrate the application to the subject. The application of the solution is exactly the same as if the study subject were to irrigate with regular saline as part of their daily regimen for chronic rhinosinusitis.

5.5 Subject Compliance Monitoring

In general, these are highly motivated patients who are seeking alternative therapy for traditional antibiotics. They have undergone surgical intervention (sometimes multiple surgeries). However, the measure of compliance will be determine by measuring the amount of solution remaining as the subjects return their irrigant bottles at the 4-week visit.

5.6 Prior and Concomitant Therapy

The vast majority of our patients are typically using intranasal steroids. This is permitted during this trial. We will ask them not to use any conventional oral, or topically applied antibiotics. If the patient feels that their infection is progressing, we would ask that they return to the clinic for evaluation at which time, the treating physician can decide to place them on standard antibiotic therapy.

5.7 Packaging

1 mg/ml concentration of quinine in saline solution will be given to participants to instill in each nostril. Product will be packaged in 90mL amber glass boston rounds, which have been autoclaved prior to use. A pharmaceutical press-in bottle adapter will be inserted into the neck and a child-resistant cap tightly placed onto the bottle. Doses will be administered by use of an LMA® MAD NASAL™ Intranasal Mucosal Atomization Device with 3mL syringe and vial adapter (Teleflex Medical, Research Triangle Park NC; item # MAD140OS), which is patient-specific. Participants will do this twice per day for 4 weeks. Subjects will clean the mucosal atomization device after each use with an alcohol swab.

Blinding of Study Drug
The placebo arm will be spiked with Sucrose Octaacetate which has a bitter taste, but does not stimulate sinonasal NO production.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Upon receipt of the of the study supplies, an inventory must be performed and a receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the delivery from IDS contains the necessary items for the protocol.

5.8.2 Storage

Solutions can be stored at room temperature in the light protected bottles they are supplied with.

5.8.3 Dispensing of Study Drug

IDS will record and dispense the quinine or placebo.

5.8.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of solution provided, consumed, and solution remaining. This reconciliation will be logged on the reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused irrigant.

6 Study Procedures

	Clinical Care	Research
Baseline Visit (Week 0)	SNOT-22	Microbiome swab(s)
	CT Scan of Sinus (if clinically indicated)	Sniffin' Stick Test
	Nasal Endoscopy	Given Saline *OR* Quinine Solution
	Sinus culture swab(s)	Taste test
Irrigate Sinus 2 Times Every Day for 4 Weeks		
4 Week Visit (+/- 7 days)	SNOT-22	Microbiome swab(s)
	Sinus culture swab(s)	Sniffin' Stick Test
	Nasal Endoscopy	Return Unused Solution

12 Week Visit (+/- 14 days)	SNOT-22	Microbiome swab(s)
	CT Scan of Sinus (if clinically indicated)	Sniffin' Stick Test
	Nasal Endoscopy	
	Sinus culture swab(s)	

6.1 Visit 1 (week 0)

Patients who consent to participate will be randomized to a treatment arm of quinine and saline (1mg/ml) or sucrose octaacetate (0.5mg/ml) in saline as dispensed by IDS. The study nurse coordinator will instruct the patient on how to administer the solution.

Following the routine endoscopically guided sinus culture swab, an additional research microbiome swab will be obtained. Patients will be asked to complete a routine Sinonasal Outcomes Test (SNOT-22) questionnaire as well as undergo a Sniffin' Stick Test as part of the research. Additionally, as part of their initial clinical assessment, a nasal endoscopy will be performed and recorded. This will later be scored by a blinded clinical investigator. If a CT scan of the sinuses is clinically indicated, then one will be performed. Additionally, a taste test to determine bitter taste sensitivity will be administered. This taste test is comprised of non-toxic solutions which are not swallowed by the patient.

6.2 Visit 2 (week 4, +/- 7 days)

Subject is asked to return to the clinic where they will be subjected to the same procedures at Visit 1. The clinical research nurse will measure and record the volume of nasal spray remaining in the stock bottle to determine compliance. No CT scan will be performed at the 2nd visit.

6.3 Visit 3 (week 12, +/- 14 days)

Subject is asked to return to the clinic where they will be subjected to the same procedures at Visit 1. A CT scan will only be performed if clinically indicated by the physician.

6.4 Sample Size Determination

There is no prior data to base a power calculation on. This trial will utilize a total of 100 patients to help guide future studies with appropriate power. In order to determine this sample size, we utilized a calculation that would ensure us that our confidence interval has a specified width. A previous study sampled over 300 CRS patients and found pre- and post-surgical SNOT-22 scores to have a standard deviation of about 20[21]. Based on this prior work, we estimate the required sample size to detect an effect with a precision of 10 (half the width of the desired 95% CI) to be 32 patients in each group. We will therefore recruit a total of n=100 patients in order to provide a margin for error, early withdrawal, or non-compliance.

6.5 Statistical Methods

Data will be analyzed after 100 subjects have completed the study. Descriptive demographic data will be compiled including mean and median age, adjuvant therapy use, use of oral antibiotics, and microbiome profile. Longitudinal linear mixed effects modeling will be used to examine the relationship between all outcome measures and treatment assignment, assuming that the distribution of the outcome measures follow a unimodal symmetric distribution and satisfies the assumptions for mixed effects modeling. Mixed effects modeling takes advantage of all available data (up to the point of loss to follow-up or withdrawal) and also can address missing data. As long as the level of missingness is not excessive and patterns of missingness are not detected, mixed effects modeling can use all available data.[21-24] For all analyses, alpha will be set to 0.05 (two-sided).

The Epidemiology Department may be consulted at the completion of the study to assist with data analysis. Only de-identified data will be provided. No PHI will be shared.

6.6 Subject Population(s) for Analysis

All-randomized population: Any subject randomized into the study, regardless of whether they received study drug, will be analyzed.

7 Safety and Adverse Events

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in inpatient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is defined as the period from the initiation of any study procedures to the end of the study follow-up.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

7.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study irrigant was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study irrigant

7.3.1 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

Unexpected

AND

Related to the research procedures

Reporting Process
Unanticipated problems posing risks to subjects will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Other Reportable events:
The following events are also reportable to the Penn IRB:

- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

7.4 Unblinding Procedures

This study will be unblinded upon completion. Once the lead investigator determines that the study recruitment is complete and ready for data analysis, he will provide IDS with a written request for study unblinding. IDS will then check allocation log for completion, sign and date the last entry, and provide the lead investigator the log of all study allocations.

In the event that unblinding is required to make a clinical treatment decisions (ie: unexpected serious adverse events), an emergency unblinding request for that particular subject will be requested by the lead investigator to IDS.

Unblinding will be required:

- To make clinical treatment decisions when an unexpected serious adverse event occurs
- During unmasked analysis in accordance with the study analysis plan
- At the request of the IRB or Office of Clinical Research
- At the conclusion of the study

7.5 Stopping Rules

The first 10 participants of the trial will be un-blinded to the attending physicians. They will monitor the clinical efficacy of the treatment, and analyze outcome measures to determine whether or not the trial should proceed or be aborted. The trial will continue if and only if no clinically significant adverse events are observed in the treatment and/or placebo arms.

7.6 Medical Monitoring

It is the responsibility of the Sponsor to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects who have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Electronic data will be stored on an institutionally secured and managed drive and/or in the Research Electronic Data Capture system (REDCap). Paper records will be stored in a locked cabinet in a secure location. Only authorized study personnel will have access.

8.2 Source Documents

Original documents, and data records include: hospital records, clinical and office charts and procedural information, laboratory notes and results, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, and records kept at the pharmacy/IDS, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

8.4 Records Retention

Study documentation will be retained for 2 years after the last-study related subject visit.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

The Investigator and Sponsor will allocate adequate time for monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

9.2 Auditing and Inspecting

The Investigator will permit study-related monitoring, audits, and inspections by the Sponsor, EC/IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

Funded through departmental academic development funds

11.2 Conflict of Interest

N/A

11.3 Subject Stipends or Payments

Subjects will not be compensated for their participation.

12 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided for the purposes of performing the study, will be published or passed on to any third party without the consent of the study PI (Nithin Adappa, MD). Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

13 References

1 Ly, N. & McCaig, L. F. National Hospital Ambulatory Medical Care Survey: 2000 outpatient department summary. *Adv Data* **327**, 1-27 (2002).
2 Bhattacharyya, N. Incremental healthcare utilization and expenditures for allergic rhinitis in the United States. *Laryngoscope* **121**, 1830-1833, doi:10.1002/lary.22034 (2011).
3 Group, M. R. ENT and Bronchoscopy Market Outlook (http://www.mrg.net, 2013).
4 Smith, S. S. *et al.* National burden of antibiotic use for adult rhinosinusitis. *J Allergy Clin Immunol* **132**, 1230-1232, doi:10.1016/j.jaci.2013.07.009 (2013).
5 Fokkens, W. J. *et al.* EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology* **50**, 1-12, doi:10.4193/Rhin (2012).
6 Peters, A. T. *et al.* Diagnosis and management of rhinosinusitis: a practice parameter update. *Ann Allergy Asthma Immunol* **113**, 347-385, doi:10.1016/j.anai.2014.07.025 (2014).
7 Bhattacharyya, N. & Kepnes, L. J. The microbiology of recurrent rhinosinusitis after endoscopic sinus surgery. *Arch Otolaryngol Head Neck Surg* **125**, 1117-1120 (1999).
8 Grobler, A. *et al.* Pre- and postoperative sinus penetration of nasal irrigation. *Laryngoscope* **118**, 2078-2081, doi:10.1097/MLG.0b013e31818208c1 (2008).
9 Wei, C. C., Adappa, N. D. & Cohen, N. A. Use of topical nasal therapies in the management of Chronic rhinosinusitis. *Laryngoscope*, doi:10.1002/lary.24066 (2013).
10 Lee, R. J., Chen, B., Redding, K. M., Margolskee, R. F. & Cohen, N. A. Mouse nasal epithelial innate immune responses to Pseudomonas aeruginosa quorum-sensing molecules require taste signaling components. *Innate immunity* **20**, 606-617, doi:10.1177/1753425913503386 (2013).
11 Lee, R. J. & Cohen, N. A. The emerging role of the bitter taste receptor T2R38 in upper respiratory infection and chronic rhinosinusitis. *Am J Rhinol Allergy* **27**, 283-286, doi:10.2500/ajra.2013.27.3911 (2013).
12 Lee, R. J. *et al.* Bitter and sweet taste receptors regulate human upper respiratory innate immunity. *J Clin Invest*, doi:10.1172/JCI72094 (2014).
13 Lee, R. J. *et al.* T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest* **122**, 4145-4159, doi:10.1172/JCI64240 (2012).
14 Lee, R. J. & Cohen, N. A. Taste receptors in innate immunity. *Cell Mol Life Sci*, doi:10.1007/s00018-014-1736-7 (2014).
15 Lee, R. J. & Cohen, N. A. Role of the bitter taste receptor T2R38 in upper respiratory infection and chronic rhinosinusitis. *Curr Opin Allergy Clin Immunol*, doi:10.1097/ACI.0000000000000120 (2014).
16 Desrosiers, M. Y. & Salas-Prato, M. Treatment of chronic rhinosinusitis refractory to other treatments with topical antibiotic therapy delivered by means of a large-particle nebulizer: results of a controlled trial. *Otolaryngol Head Neck Surg* **125**, 265-269, doi:10.1067/mhn.2001.117410 (2001).
17 Ramsey, B. W. *et al.* Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N Engl J Med* **328**, 1740-1746, doi:10.1056/NEJM199306173282403 (1993).
18 Psaltis, A. J., Li, G., Vaezeafshar, R., Cho, K. S. & Hwang, P. H. Modification of the Lund-Kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. *Laryngoscope* **124**, 2216-2223, doi:10.1002/lary.24654 (2014).
19 Hopkins, C., Browne, J. P., Slack, R., Lund, V. & Brown, P. The Lund-Mackay staging system for chronic rhinosinusitis: how is it used and what does it predict? *Otolaryngol Head Neck Surg* **137**, 555-561, doi:10.1016/j.otohns.2007.02.004 (2007)
20 Delwiche, J. F., Buletic, Z. & Breslin, P. A. Covariation in individuals' sensitivities to bitter compounds: evidence supporting multiple receptor/transduction mechanisms. *Percept Psychophys* **63**, 761-776 (2001).
21 Diggle P, Heagerty P, Liang K-Y, Zeger S. Analysis of longitudinal data: Oxford University Press; 2002.
22 Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982:963-74.
23 Maldonado G, Greenland S. Simulation study of confounder-selection strategies. American journal of epidemiology 1993; 138:923-36.

24 Zeger SL, Liang K-Y. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986:121-30.