Phase Ib/II Clinical Trial of Topical Verapamil HCl for Chronic Rhinosinusitis with Nasal Polyps

Protocol Identifying Number: 17-002H

Principal Investigator: Benjamin Bleier, MD

IND/IDE Sponsor: N/a

Funded by:

Draft or Version Number: 2.31

06 June 2017

Table of Contents

LIST OF ABBREVIATIONS

AE	Adverse Event
BID	Twice a day
BMI	Body Mass Index
BP	Blood Pressure
CRF	Case Report Form
CRS	Chronic Rhinosinusitis
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
EKG	Electrocardiogram
ELISA	Enzyme Linked Immunosorbent Assay
FDA	Food and Drug Administration
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GCP	Good Clinical Practice
HCl	Hydrochloride
IHC	Immunohistochemistry
IL	Interleukin
IN	Intranasal
IND	Investigational New Drug
IRB	Institutional Review Board
ICC	Intraclass Correlation Coefficient
IT	Intermediate Toxicity
LMK	Lund-Mackay
LKS	Lund-Kennedy Score
LSM	Least Squares Mean
MAD	Maximal Administered Dose
MAP	Mean Arterial Pressure
MCID	Minimally Clinically Important Difference
MEEI	Massachusetts Eye and Ear Infirmary
MTD	Maximal Tolerated Dose
PBS	Phosphate Buffered Saline
PCFD	Partition Coefficient of the Free Drug
P-gp	P-glycoprotein
PI	Principle Investigator
PVA	Polyvinyl Alcohol Sponge
SA	Specific Aims
SNOT-22	Sinonasal Outcomes Test (22-item)
sNP	Without Nasal Polyps
SEB	Staphylococcal aureus enterotoxin B
TEM	Transmission Electron Microscopy
Th2	Type 2 Helper T-cell
TID	Three Times a Day
TSLP	Thymic Stromal Lymphoprotein
UCF	Ultracentrifugation
wNP	With Nasal Polyps
VAS	Visual Analog Scale
Zos	Zosuquidar Trihydrochloride

STATEMENT OF COMPLIANCE

This trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the <NIH IC> Terms of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: _____
Print/Type Name

Signed: _____ Date: _____

PROTOCOL SUMMARY

Title: Phase Ib/II Clinical Trial of Topical Verapamil HCl for Chronic Rhinosinusitis with Nasal Polyps

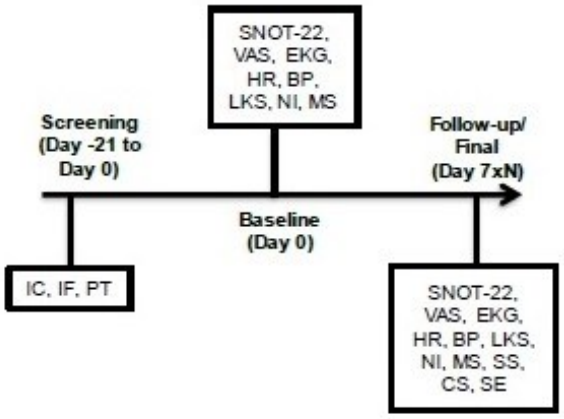
Précis: Phase Ib will be an accelerated titration, intrapatient dose escalation cohort, with double-dose step design. Participants will administer each dose Verapamil HCl dose intranasally BID. Weekly visits will occur for side-effect and escalation evaluations. Phase II will be an open label trial of 20 participants. The MTD determined in Phase Ib will be administered BID for 4 weeks. Participants will be seen at baseline, 1 week, and 4 week for efficacy and side-effect evaluations.

Objectives:
Primary Objectives:
-Perform a Phase IB trial of topical intranasal Verapamil HCl BID to determine the Maximal Tolerated Dose(MTD) using an accelerated titration design with intra-patient dose escalation.
-Perform a Phase II trial of topical intranasal Verapamil HCl BID based on the Phase I MTD to determine safety and efficacy using both subjective and objective outcome measures of CRS.
Secondary Objective:
-Determine whether mucus derived exosomal P-gp can be used as a non-invasive biomarker of CRS by correlating it with baseline symptoms of CRSwNP and treatment response to topical Verapamil HCl at the MTD.

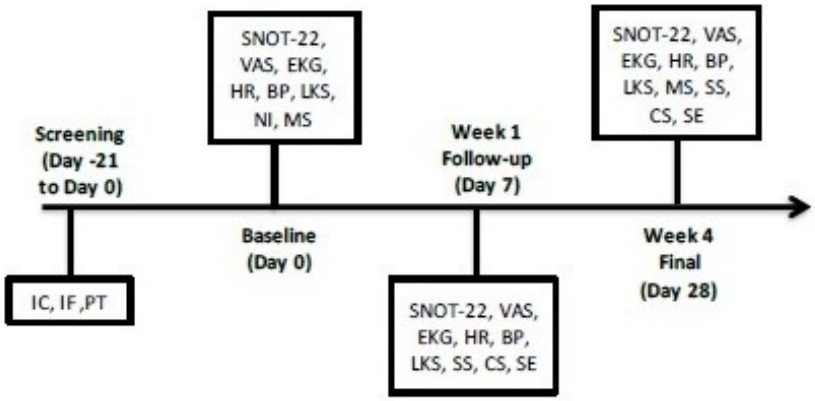
Endpoint
Primary Endpoints:
Phase Ib: Defining the Maximal Tolerated Dose(MTD) of topical Verapamil HCl. DLT will be defined as a development of 2nd or 3rd degree heart block as measured by an EKG.
Phase II: determining the efficacy of topical Verapamil HCl at the MTD for subjective symptoms of CRSwNP using the validated SNOT-22.
Secondary Endpoints:
Phase Ib: Intermediate toxicity will be defined as: a heart rate of <50, an asymptomatic BP reduction >30% from baseline or SBP <90mmHg, an asymptomatic AMP reduction >30% from baseline or MAP<55, an asymptomatic DBP reduction >30% from baseline, and a Meltzer Compliance Grade >4.

	Phase II: to determine the efficacy of Verapamil HCl at the MTD as measured by a subjective 10cm visual analog scale(VAS), Meltzer topical irrigation satisfaction and compliance scores, and the validated Lund-Kennedy nasal endoscopy score(LKS).
Population:	20-40 MEE Sinus Center patients over the age of 18 who present with chronic rhinosinusitis with polyps.
Phase:	IB/II
Number of Sites enrolling participants:	1
Description of Study Agent :	Verapamil HCl 0.3mg-3.6mg final dose BID in buffered normal saline as a nasal irrigation.
Study Duration:	December 2016 – December 2018
Participant Duration:	1 month

SCHEMATIC OF STUDY DESIGN



Legend	
IC	Informed Consent
IF	Intake Form
PT	Pregnancy Test
SNOT-22	Sino-Nasal Outcome Test
VAS	Visual Analogue Scale
EKG	Electrocardiogram
HR	Heart Rate
BP	Blood Pressure
LKS	Lund-Kennedy Score
NI	Nasal Irrigation
MS	Mucus Sponge
SS	Satisfaction Scale
CS	Compliance Scale
SE	Side-Effects



1 KEY ROLES

Name, degree	Institution	Address	Phone Number	Email
Benjamin Bleier, MD	MEEI	243 Charles St, Boston, MA 02114	617-573-6966	Benjamin_Bleier@meei.harvard.edu
Patricia Levesque, BS	MEEI	243 Charles St, Boston, MA 02114	617-573-3576	Patricia_levesque@meei.harvard.edu
Sarina Mueller, MD	MEEI	243 Charles St, Boston, MA 02114	n/a	Sarina_mueller@meei.harvard.edu
Jeremy Goldfarb, MD	MEEI	243 Charles St, Boston, MA 02114	617-573-3380	Jeremy_goldfarb@meei.harvard.edu
Christine Finn, Pharm-D	MEEI	243 Charles St, Boston, MA 02114	617-573-3315	Christine_finn@meei.harvard.edu
Angela Nocera, MS	MEEI	243 Charles St, Boston, MA 02114	n/a	Alnocera5@gmail.com
Hang Lee, Ph.D.	MGH Biostatistics Center	50 Staniford St, Boston, MA 02114	617-726-4293	Hlee5@mgh.harvard.edu

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

P-glycoprotein is overexpressed in Th2 polarized endotypes of CRS and correlates with indices of disease severity. Previous studies by our group have demonstrated that P-gp is differentially overexpressed in the sinus mucosa of patients with CRSwNP relative to CRS without Nasal Polyp(CRSsNP) and control patients. This overexpression is specific to the sinus mucosa as the adjacent non-polypoid septum has similar expression levels across all patient groups [10]. We next focused on the CRSsNP population to determine whether P-gp expression correlated with clinical severity. We found that high P-gp expression patients with CRS had greater tissue eosinophilia and worse CT inflammatory scores than their low P-gp expression CRSsNP counterparts. These findings indicate that P-gp is overexpressed in CRSwNP and eosinophilic CRSsNP, both of which are associated with Th2 polarized inflammation.

P-gp overexpression in CRSwNP directly leads to Th2 cytokine hyper-secretion. Having demonstrated that P-gp is overexpressed in Th2 polarized forms of CRS(CRSwNP and eosinophilic CRSsNP), we next studied whether this overexpression is associated with increased secretion of cytokines which promote Th2 inflammatory pathways. We utilized an organotypic explant model to confirm that P-gp is overexpressed in CRSwNP relative to non-eosinophilic CRSsNP. Using this explant model we showed that P-gp directly promotes the secretion of both IL-5 and thymic stromal lymphopoietin (TSLP). Furthermore, the degree of secretion of these important Th2 polarizing cytokines was strongly and significantly correlated with P-gp expression within the same explant[45]. These results confirm previous in vitro findings by our group in polyp derived epithelial cell cultures[44]. Taken together, these results demonstrate that P-gp mediated cytokine hyper-secretion plays a critical role in the pathogenesis of Th2 polarized forms of CRS including CRSwNP.

Mucus derived exosomal P-gp is a non-invasive biomarker of CRSwNP. Our group recently reported on the presence of a secreted form of P-gp which could be detected in nasal mucus. While the presence of P-gp in mucus is physiologic, we showed that total P-gp concentrations above 250 pcg/µg of total protein are associated with CRSwNP as well as more severe subjective and objective findings of disease[13]. We subsequently discovered that this secretion was mediated by the release of exosomes which are differentially enriched with P-gp among patients with CRSwNP. In light of the importance of P-gp overexpression to the pathogenesis of CRSwNP, the development of a non-invasive test of expression which intrinsically resists degradation and can be correlated with disease severity would be extremely valuable.

Verapamil HCl is an effective P-gp inhibitory therapy for CRSwNP. However well tolerated oral doses are subtherapeutic in patients with elevated BMI. The significant contribution of P-gp to the pathogenesis of CRSwNP suggests that it may represent a novel druggable target. We therefore undertook a randomized, double-blind, placebo-controlled trial to test the efficacy of low dose oral Verapamil HCl, a known first generation P-gp inhibitor[14], for the treatment of CRSwNP[19]. While Verapamil is cardioactive, it is considered a first-line prophylactic drug for cluster headache and is well tolerated at 80mg three times a day(TID) by otherwise healthy patients[51]. Our findings demonstrated significant efficacy in both our primary and secondary endpoints with no significant side effects. However, a logistic regression analysis revealed two important relationships between baseline characteristics and efficacy. First, patients with elevated BMI had significantly lower improvements in SNOT-22($p=0.01$). This is consistent with our use of a low dose of a relatively low potency inhibitor. The second is that patients with the highest total mucus P-gp levels experienced less benefit($p=0.01$). This strongly suggests that the mechanism of Verapamil is acting through P-gp inhibition and that patients with greater expression may need higher concentrations to achieve adequate pump suppression. While Verapamil HCl has significant potential for the treatment of CRSwNP through P-gp inhibition, higher doses must be achieved to extend the effect to patients with elevated BMIs and the highest levels of P-gp expression. As increasing oral dosing could result in cardiac side effects, topical delivery represents a promising alternative. As exosome bound P-gp may be more stable and representative of disease state than total mucus P-gp concentration, exosomal P-gp demands further exploration as a novel biomarker of disease severity and drug response.

Pharmacokinetic comparison of oral versus topical Verapamil administration. The bioavailability factor of Verapamil HCl has been estimated at 29-47.8% intranasal(IN) and 20-35% orally[52][53]. Due to the stereoselective first-pass effect of oral Verapamil, the dextro/levo isomer ratio should be taken into account indicating that 2-3 times higher Verapamil concentrations are needed to obtain a similar effect after oral versus IN administration [54]. The ratio of the IN/oral bioavailability divided by the stereoselective effect suggests that an IN dose of 11.2-48.5mg of Verapamil would be equivalent to a single 80mg oral dose. To achieve the same exposure effect at steady state TID dosing, the equivalent BID IN dose would therefore be 16.8-72.75mg. The partition coefficient of the free drug(PCFD), the unbound fraction capable of diffusing into the mucosa, has been estimated to be between 2.0-8.0[55][56][57]. Based on the mucosal volumetric analysis by Sarangapani et al[58], the PCFD of Verapamil, and

our previous ¬in vitro data[48], the minimal peak IN dose required to achieve an adequate suppression of P-gp by Verapamil would be 0.03mg.

2.2 RATIONALE

Treatment of CRSwNP poses a significant challenge as the mechanisms responsible for initiating and maintaining Th2 inflammation are poorly understood. Consequently, the development of cost effective, targeted therapies, with limited off target toxicity remains a significant unmet need. This knowledge gap has also hindered the discovery and validation of novel biomarkers which could be used to predict disease endotype and prognosis. This proposal is based on several recent discoveries suggesting that P-glycoprotein(P-gp) functions as a key immunomodulator of Th2 cytokine secretion in CRSwNP. The P-gp hypothesis of CRSwNP has yielded both a novel druggable target as well as a possible non-invasive biomarker of disease severity and treatment response. The successful completion of this proposal therefore has the potential to significantly shift the way we diagnose and treat CRSwNP.

CRSwNP is a prevalent disease associated with major direct and indirect costs. Acute and Chronic Rhinosinusitis are estimated to affect up to 16% of the US population. They account for approximately 11 million or 1% of all office visits per year in the US and are the most common cause for antibiotic prescriptions in the community[20] [21]. CRS alone impacts more than 30 million Americans resulting in $6.9 to $9.9 billion in annual healthcare expenditures [22,23] and $12.8 billion in productivity costs[24]. The subset of patients in Europe with CRSwNP has been estimated to be between 2 and 4.3% [25][26][27] and is thought to be similar in the US. This population remains one of the most challenging subgroups of CRS to manage effectively.

There is a significant lack of innovative therapeutic strategies in CRSwNP. This is due, in large part, to a lack of clear understanding of the pathways involved in the initiation and maintainance of the inflammation characteristic of the disease. While corticosteroids remain the best studied and most effective treatment[28][29][30][31], they are non-targeted and are therefore associated with significant off target effects. Even when applied topically, steroids may be absorbed systemically[2] and their efficacy has been questioned in several clinical trials[32][33]. Currently, the largest body of literature exploring the pathogenesis of CRSwNP has focused on Th2 polarizing inflammation as a key feature of the disease process[34][35]. This has catalyzed the exploration of monoclonal antibody therapies which target specific Th2 promoting cytokines in CRS with variable clinical success.

Biologic therapies have had mixed results and are associated with significant drawbacks. Two anti-Interleukin(IL)-5 agents Reslizumab[36] and Mepolizumab[37] have been studied in the context of CRSwNP. While improvement was demonstrated among certain objective signs of inflammation, patient symptoms scores failed to significantly improve. Dupilumab, a fully human monoclonal antibody to the IL-4 receptor α subunit that inhibits both IL-4 and IL-13 signaling has also recently been studied[38] [3]. The most recent study compared patients with

CRSwNP treated with placebo to Dupilumab for 16 weeks. This study demonstrated a significant improvement in SNOT-22 of −18.1 in the Dupilumab group however significant treatment related side effects were reported including a 47% rate of nasopharyngitis and 40% rate of injection site reactions. While the outcomes are promising, the need for weekly injections, significant side effects, high cost, and potential for loss of efficacy over time suggest that the development of alternative, cost effective, targeted non-steroidal therapeutic strategies are still needed to optimize treatment of CRSwNP.

There is an unmet need for the discovery and validation of non-invasive biomarkers of CRSwNP. As personalized treatment of CRS continues to advance, the development of biomarkers of disease and endotype will become critical. A recent study by Tomassen et al[39] provided a proof of concept that an unsupervised partition based cluster analysis of tissue derived cytokines and inflammatory proteins could yield discreet clusters which correlated well with phenotypic characteristics. Despite the promise of this technique, the biomarkers studied required tissue sampling which was invasive, introduced the possibility of inter-patient heterogeneity in tissue composition, and protein degradation during the sampling and storage process. Furthermore, the inevitable medical therapies leading up to the surgical event at which the sampling occurred were not accounted for in the analysis. Consequently, a novel method of biomarker sampling which can be performed non-invasively in the outpatient setting prior to the initiation of medical therapy is needed. Ideally these biomarkers would directly participate in, and thereby closely reflect, the underlying pathophysiology and would be intrinsically resistant to degradation.

Mucus derived exosomes represent an ideal potential biomarker reservoir. Exosomes are 30–150 nm vesicles surrounded by a lipid bilayer that are released from epithelial cells into nasal mucus[40][41] [42]. Biophysically, exosomes are equivalent to cytoplasm and carry integral membrane proteins, mRNA, and regulatory microRNA reflective of their cell of origin. Studies have demonstrated that exosomes are able to shuttle their cargo to adjacent cells where they can exert a paracrine regulatory function on the recipient cell[43]. The tightly regulated composition of exosomes, their presence in nasal mucus, and their protective lipid bilayer make them a potentially ideal source of biomarkers for outpatient sampling which could out-perform total dissolved protein levels given their vulnerability to proteolytic degradation.

The discovery of a new pathogenic mechanism for CRSwNP which yields both novel targeted therapeutics and non-invasive biomarkers of disease would represent an important and significant advance in the field of Rhinology.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Verapamil HCl is a cardioactive drug and thus there is a theoretical risk of cardiac side effects from the medication. This will be mitigated by obtaining a EKG which will be reviewed by a medical internist/anesthesiologist prior to initiating therapy. Follow-up EKGs will also be performed to monitor for cardiac side effects. The risk of cardiac effects appears to be dose-dependant and is expected to be mostly reversible upon discontinuation of the drug. Additional risks include bradycardia and hypotension. These will be mitigated by checking routine vital signs at each visit which will include heart rate and blood pressure. Constipation is also a risk of the drug. This will be asked about at each visit and stool softeners and/or laxatives will be administered as necessary. Since verapamil will be administered with a nasal rinse there is a possibility of nasal irritation. These side effects will be monitored at each study visit.

There are currently few medical interventions available for CRSwNP. The development of new medications is critical to the management of this disease. Thus, the short-term risks for the trial patients will ultimately decrease the risks and discomforts that the overall CRSwNP population experiences.

2.3.2 KNOWN POTENTIAL BENEFITS

If successful, using Topical Verapamil will help treat pateints with CRSwNP and potentially enable them to avoid sinus surgery or steroid therapy with their attendant risks.

Successful performance of Topical Verapamil will establish this drug as a novel therapeutic option for the treatment of CRSwNP as well as elucidate a mechanism of the pathophysiology of CRSwNP. This may also open avenues for the use of a new class of treatments for CRSwNP which work along the same mechanism.

3 OBJECTIVES AND PURPOSE

Primary Objectives:

- Perform a Phase IB trial of topical intranasal Verapamil HCl BID to determine the Maximal Tolerated Dose(MTD) using an accelerated titration design with intra-patient dose escalation.
- Perform a Phase II trial of topical intranasal Verapamil HCl BID based on the MTD to determine safety and efficacy using both subjective and objective outcome measures of CRS.

Secondary Objective:

- Determine whether mucus derived exosomal P-gp can be used as a non-invasive biomarker of CRS by correlating it with baseline symptoms of CRSwNP and treatment response to topical Verapamil HCl at the MTD.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

ARM 1: Phase 1B

The phase IB study will consist of an accelerated titration, intrapatient dose escalation cohort, with double-dose step design. The initial single patient cohort will begin by taking 0.3mg (10mg of Verapamil in 240mL of buffered normal saline yielding an established 3% residual nasal dose) as a nasal irrigation BID for 1 week. The first dose f

each escalation will be administered in a 10th or 11th floor MEE clinic withhemodynamic monitoring. A medically trained member of the study staff with monitor the patient for one hour. Patients will be instructed on the how to properly perform the irrigation using a pre-recorded video demonstration. If no first-course dose limiting toxicity (DLT, defined by the development of 2nd or 3rd degree heart block) is noted then patients will continue taking the current Verapamil rinse dose BID for 1 week. Dose escalation will occur weekly in the absence of a single, any course, DLT or a second, any course, intermediate toxicity(IT, defined by a heart rate of <50, an asymptomatic BP reduction >30% from baseline or systolic BP <90mmHg, an asymptomatic MAP reduction >30% from baseline or MAP<55, an asymptomatic diastolic BP reduction >30% from baseline, and a Meltzer Compliance Grade >4[60]). Each escalation will be a doubling of the dose from 0.3-2.4mg. At that point that dose will escalate in 0.6mg residual intervals for the rest of the trial up to a maximum of 3.6mg total residual dose. These doses were derived from the pharmacokinetic analysis of our oral Verapamil trial results. If a single, any course, DLT or second, any course, IT occurs, two additional patients will be recruited at that identified dose and Phase IB will revert to a standard 3+3 design. If any patient un-enrolls while the dose escalation is still occurring, they will be replaced to maintain 3 patient cohorts. The maximal administered dose(MAD) will be considered that at which at least 2 DLTs or 4 ITs occur and the MTD will then be assigned to the immediate preceeding dose.

At each weekly visit, the patients nasal endoscopy will be recorded for LKS grading, and they will be administered symptom, side-effect, and tolerability questionnaires. Pre-dosing mucus and post-dosing irrigant samples will also be atraumatically collected from the nasal cavity for use in exosomal biomarker sampling.

ARM 2: Phase 2

The Phase II study will be an open label safety and efficacy expansion cohort using the MTD determined in the Phase IB arm. A total of 20 patients will be administered the MTD of topical Verapamil HCl in a 240mL buffered normal nasal rinse for 4 weeks BID. This sample size was calculated based on a power analysis derived from the results of our oral Verapamil trial. The first dose will be administered in in a 10th or 11th floor MEE clinic withhemodynamic monitoring. A medically trained member of the study staff with monitor the patient for one hour. Patients will be instructed on the how to properly perform the irrigation using a pre-recorded video demonstration. If no first-course DLT occurs then patients will continue taking the topical Verapamil dose BID. Patients will then return for follow-up visits at 1 week and 4 weeks. Subjective and objective outcome measures will be collected at each visit.

4.2.1 PRIMARY ENDPOINT

Phase Ib:

The primary outcome measurement for Phase IB(SA1) will be defining the Maximal Tolerated Dose(MTD) of topical Verapamil HCl. Dose Limiting Toxicity will be defined as a development of 2nd or 3rd degree heart block as measured by an EKG.

Phase II:

The primary outcome for Phase II(SA2) will be determining the efficacy of topical Verapamil HCl at the MTD for subjective symptoms of CRSwNP using the validated SNOT-22.

4.2.2 SECONDARY ENDPOINTS

Phase Ib:

Intermediate toxicity will be defined as: a heart rate of <50, an asymptomatic BP reduction >30% from baseline or SBP <90mmHg, an asymptomatic AMP reduction >30% from baseline or MAP<55, an asymptomatic DBP reduction >30% from baseline, and a Meltzer Compliance Grade >4.

Mild toxicity will be defined as a Meltzer Compliance Grade of 2-3.

Phase II:

The secondary outcomes of Phase II will be to determine the efficacy of Verapamil HCl at the MTD as measured by a subjective 10cm visual analog scale(VAS), Meltzer topical irrigation satisfaction and compliance scores, and the validated Lund-Kennedy nasal endoscopy score(LKS).

4.2.3 EXPLORATORY ENDPOINTS

Phase Ib:

The efficacy of Verapamil HCl irrigations in the treatment of CRSwNP using validated symptoms outcome test (SNOT-22) and a 10cm visual analogue scale (VAS).

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

Phase 1 Inclusion:

- Patients presenting to the MEEI Sinus Center
- Age 18-80 yrs old
- Diagnosed with Chronic Rhinosinusitis with Nasal Polyps according to the EPOS 2012 consensus criteria

Phase 2 Inclusion

- Post-operative with a Lund-Kennedy Poly score of <4
- Baseline SNOT-22 Score ≥ 30
- Patients presenting to the MEEI Sinus Center
- Age 18-80 yrs old
- Diagnosed with Chronic Rhinosinusitis with Nasal Polyps according to the EPOS 2012 consensus criteria

5.2 PARTICIPANT EXCLUSION CRITERIA

Patients with the following comorbidities:

•GI Hypomotility

•Heart Failure

•Liver Failure

•Kidney Disease

•Muscular Dystrophy

•Pregnant or Nursing Females

•Systemic Steroid Dependency

•Hypertrophic Cardiomyopathy

•Any Atrial or Ventricular arrhythmia (ie. Atrial fibrillation, atrial flutter, etc..)

•Resting Heart Rate less than 60 beats per minute

•Baseline Systolic Blood Pressure less than 110 mmHg

•Baseline Diastolic Blood Pressure less than 70 mmHg

•Baseline Mean Arterial Pressure Less than 60 mmHg

•PR interval less than 0.12 seconds

Patients taking the following medications:

•Aspirin

•Beta-blockers

•Cimetidine(Tagamet)

•Clarithromycin(Biaxin)

•Cyclosporin

•Digoxin

•Disopyramide(Norpace)

•Diuretics

•Erythromycin

•Flecainide

•HIV Protease Inhibitors(Indinavir, Nelfinavir, Ritonavir)

•Quinidine

•Lithium

•Pioglitazone

•Rifampin

•St Johns Wort

Patients who are unwilling or unable to wash out of systemic steroids

Patients with cardiac or conduction abnormality picked up by screening EKG

Post-op patients with surgery within 3 months prior to enrollment.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

A concerted effort will be made to recruit male and female subjects equally. Special attention will also be paid to enroll minority subjects into this trial to represent the ethnic demographics of the region as closely as possible. No sex/gender or racial/ethnic group will be excluded from this study.

Patients in the Phase 1B arm will be compensated $150 per visit at the end of their participation to compensate them for the frequent nature of the visits. Patients in the Phase 2 arm will be compensated $150 for their participation at the end of their week 4 visit.

All subjects will be recruited from the MEEI Sinus Center. The MEEI Sinus Center has over 3000 outpatient visits per year. This should provide a more than adequate patient pool to recruit from. Patients will be informed of the Phase 1B or Phase 2 trial during a regular clinical visit. If they express interest in the trial the clinical coordinator will explain the parameters of the study and review inclusion/exclusion criteria.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Arm 1: Phase 1B

The PI will terminate a Phase IB participant if they experience a DLT, more than one IT, or begin a contradictory medication during the course of the trial. Patients may withdraw from the trial at any time upon request. Any withdrawn participants from Phase IB will be replaced to maintain 3 patient cohorts. Their reason for withdrawal will be noted.

Arm 2: Phase 2

The PI will terminate a Phase II study participant if they present with any clinical adverse event (AE) or other medical complication such that continued participation in the study would not be in the best interest of the participant. The PI will terminate a participant if they miss more than 2 doses of topical Verapamil in a week. Compliance will be checked using a patient dosing diary. Participants may withdraw from the trial at any time upon request. Any withdrawn participant will be replaced with a new enrollment.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Patients that withdraw or are terminated from either arm of the study due to an adverse event will be followed for up to 1 year to monitor for additional safety data.

Phase 1B: Any withdrawn participants will be replaced to maintain 3 patient cohorts.. Their reason for withdrawal will be noted.

Phase 2: Any withdrawn participant will be replaced with a new enrollment.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigator. IIf the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants including a greater than 20% AE rate
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the sponsor, IRB and/or FDA.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

The Verapamil HCl irrigations will be acquired from the MEEI Pharmacy. Christine Finn, PharmD will be in charge of the prepareation of each dose.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Phase Ib
Verapamil solution for injection, supplied in vials, will be utilized for this phase of the trial. Verapamil solution for injection in vials is commercially available through the Pharmacy's wholesaler (Cardinal Health). Depending on specific product availability from the wholesaler, one of the following verapamil solution for injection in vial will be used:

- Verapamil 10mg/4mL vial (NDC 00409-1144-02, manufacturer = Hospira) **This is the preferred product if available in sufficient quantity from the wholesaler.**
- Verapamil 5mg/2mL vial (NDC 00409-1144-05, manufacturer = Hospira)
- Verapamil 5mg/2mL vial (NDC 51754-0203-02, manufacturer = Exela Pharma Sciences)

Verapamil is not available for use via the nasal irrigation route and must be formulated in Neil Med Sinus Rinse to be utilized as an irrigation.

Neil Med Sinus Rinse is commercially available through the Pharmacy's wholesaler (Cardinal Health). The Neil Med Sinus Rinse starter kit (with bottle and 5 packets, NDC 05928-0003-08, manufacturer = NeilMed Products, Inc.) and Neil Med Sinus Rinse refill packets (NDC 05928-0002-00, manufacturer = Neil Med Products, Inc.) will be used.

Please see attached package inserts (included in appendix) for more information.

6.1.3 PRODUCT STORAGE AND STABILITY

Verapamil in its commercially available form (vials or syringes) is stored at controlled room temperature (15-30 degrees C) and protected from light in its original container. Verapamil commercially available vials and syringes are single use products; if any extra medication remains in the original container after preparing the patient-specific dose it will be discarded. Verapamil will be stored in the Pharmacy in a limited access area and segregated from standard pharmacy stock; it will be specifically allocated as a supply for this trial. When patient-specific verapamil doses are prepared, the beyond-use dating will be 9 days at refrigerated temperature (2-8 degrees C)

Neil Med Sinus Rinse in its commercially available form is stored at controlled room temperature (15-30 degrees C). Neil Med packets are for single use only. Neil Med Sinus Rinse will be stored in the Pharmacy in a limited access area and segregated from standard pharmacy stock; it will be specifically allocated as a supply for this trial. When patient-specific verapamil doses are combined with Neil Med Sinus Rinse, the final product should be used immediately..

6.1.4 PREPARATION

Phase Ib

Patient-specific verapamil doses will be drawn up from the commercially available verapamil vials utilizing aseptic technique in an ISO Class 5 biological safety cabinet in the Pharmacy. At the time of use, patients will prepare the Neil Med sinus rinse from the packet according to the manufacturer's directions for preparation. The patient will transfer the verapamil from the Pharmacy-supplied syringe into the bottle containing the Neil Med sinus rinse and use immediately for nasal irrigation.

Phase II

Patient-specific verapamil doses will be provided in commercially available verapamil syringes. At the time of use, patients will prepare the Neil Med sinus rinse from the packet according to the manufacturer's directions for preparation. The patient will transfer the verapamil from the Pharmacy-supplied syringe into the bottle containing the Neil Med sinus rinse and use immediately for nasal irrigation..

6.1.5 DOSING AND ADMINISTRATION

Phase Ib:

Phase Ib participants will receive dosing according to the included escalation schedule. Dosing will go from an initial 0.3-3.6mg total residual dose of Verapamil HCl. Phase II Participants will receive the MAD determined by Phase Ib. Participants will administer the irrigation BID. A video demonstration on proper administration of the irrigation will be shown at the initial study visit.

6.1.6 ROUTE OF ADMINISTRATION

The Verapamil HCl will be administered intranasally.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

Phase Ib Verapamil HCl dose will start at a total residual dose of 0.3mg. Dose escalations will be as follows: 0.3mg, 0.6mg, 1.2mg, 2.4mg, 3.0mg, 3.6mg. Participants will remain on a dose for at least one week before escalating.

Phase II dose will be determined by Phase Ib.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

The Phase Ib dose will only be escalated if a participant has not experienced a DLT or 2 ITs on the previous dose. The Phase II dose will not be changed at any point during the 4 week course.

6.1.9 DURATION OF THERAPY

Phase Ib participants will undergo therapy for at least 1 week. Total therapy time will be determined by the number of dose escalations a participant is given.

Phase II participants will undergo therapy for 4 weeks.

6.1.10 TRACKING OF DOSE

Participants will fill out a dosing calendar which will be checked by study staff at each visit.

6.1.11 DEVICE SPECIFIC CONSIDERATIONS

N/a

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

Verapamil solution for injection and Neil Med sinus rinse will be ordered and received from the Pharmacy's wholesaler Cardinal Health in an amount sufficient to provide dosing in accordance with the subject enrollment. Verapamil and Neil Med sinus rinse will be stored in the pharmacy segregated as stock for this study only

Separate perpetual inventories will be maintained by the Pharmacy on Drug Accountability Logs for both the verapamil and Neil Med sinus rinse (see template log attached). Transactions including receipt of medication, use of medication for compounding dose / dispensation of medication to the subject, and expiration / waste of medication will be documented. In addition, a perpetual line balance indicating the number of containers of medication on hand will be documented.

Verapamil and Neil Med sinus rinse will be dispensed from the Pharmacy for a study subject pursuant to the receipt of a study medication prescription form specific to this project.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

Phase 1B: The below procedures are considered non-standard of care and are study specific:

- Meltzer Satisfaction and Compliance assessment
- Collection of mucus sample from the nasal cavity
- Weekly clinical examination
- EKG

Phase 2: The below procedures are considered non-standard of care and are study specific:

- Meltzer Satisfaction and Compliance assessment
- Collection of mucus sample from the nasal cavity
- EKG

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Phase 1B: The below procedures are considered routine standard of care in the management of patients with CRSwNP:

- Nasal Endoscopy with scoring
- SNOT-22 and VAS assessment for disease severity

Phase 2: The below procedures are considered routine standard of care in the management of patients with CRSwNP:

- Nasal Endoscopy with scoring
- SNOT-22 and VAS assessment for disease severity
- Clinic examination every 2-4 weeks in patients with CRSwNP

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Screening: A pregnancy test will be done within 24 prior to the first application of topical verapamil in women of child bearing age.

7.2.2 OTHER ASSAYS OR PROCEDURES

Exosome Purification: Mucus samples will be extracted from the PVA sponge by centrifugation (1500g at 4˚C for 30 minutes). The mucus and irrigant samples will then be diluted in 150 µL of 1x phosphate buffered saline (PBS, Life Technologies, Carlsbad, CA) with Protease Inhibitor Cocktail (1:100, Sigma, St. Louis, MO). A portion of the whole mucus sample will be saved for cytokine analysis. Exosomes will then be isolated by ultracentrifugation(UCF) as described by Théry et al[63]. Cellular debris will be pelleted by centrifugation at 45 min at 12,000 × g at 4°C. The supernatant will then be suspended in 4.5mL of PBS in polypropylene tubes (Thinwall, 5.0 mL, 13 x 51 mm, Beckman Coulter, Indianapolis, IN) and ultracentrifuged for 2 hours at 110,000 × g, at 4°C. The supernatant will then be collected and the pellet will be resuspended in 4.5 mL 1x PBS. The suspension will be filtered through a

0.22-µm filter (Fisher Scientific, Pittsburgh, PA) and collected in a fresh ultracentrifuge tube. The filtered suspension will then be centrifuged for 70 min at 110,000 × g at 4°C. The supernatant will then be collected and the pellet will be resuspended in 200 µl PBS with protease inhibitor. The exosome concentration of each pellet will be determined using a commercially available enzyme linked immunosorbent assay(ELISA) for the established exosome markers CD63 and CD9(ExoELISA, System Biosciences, Palo Alto, CA) as previously described[64].

Transmission Electron Microscopy(TEM): The presence of intact exosomes after UCF will be verified by TEM as previously described(Figure 3.5). Isolated exosomes will be fixed for 1 hour at room temperature in 2% paraformaldehyde in 0.1M sodium phosphate buffer (Electron Microscopy Sciences, Hatfield, PA). 5 µL of the exosomes will be absorbed on to Formvar-carbon coated electron microscopy grids (Electron Microscopy Sciences) for 20 minutes. After absorption, the grids will be rinsed in PBS 3 times and then transferred to PBS/50 mM glycine (Sigma Aldrich, St. Louis MO) for 4 washes. The grids will then be blocked in 5% Bovine Serum Albumin (BSA, Fisher Scientific) in 1x phosphate buffered saline (buffer) for 10 minutes at room temperature. The grids will then be incubated at 4ºC overnight in the primary antibody (1:25, Purified Mouse Anti-Human CD63 Clone H5C6, BD Biosciences) diluted in 1% BSA buffer. The grids will then be rinsed in 0.1% BSA buffer and then 0.5% BSA buffer 6 times each. Then the secondary Protein-G antibody (1:20 in 1%BSA buffer, EM Grade, 10nm, Electron Microscopy Services, Hatfield, PA) in 5% BSA buffer will be applied for 1 hour at room temperature and rinsed 8 times with 1x PBS. The grids will be incubated in 1% glutaraldehyde in 0.1M sodium phosphate buffer (Electron Microscopy Services) for 5 minutes. After rinsing 8 times in deionized water, the grids will be contrasted in uranyl-oxalate solution, pH 7 (UA, Electron Microscopy Services) for 5 minutes. The grids will be blotted on filter paper and air dried prior to imaging. The exosomes will be observed using a FEI Tecnai G2 Spirit transmission electron microscope (FEI, Hillsboro, Oregon) at an accelerating voltage of 100 kV interfaced with an AMT XR41 digital CCD camera (Advanced Microscopy Techniques, Woburn, Massachusetts) for digital TIFF file image acquisition. Rabbit IgG (Vector Laboratories, Burlingame, CA) and CD63 lysate (Novus Biologicals CD63 Overexpression Lysate (Native), Fisher Scientific) will be used as negative and positive controls, respectively.

Exosomal P-gp, Whole Mucus, and Irrigant Th2 Cytokine Quantification: Whole mucus and exosomal P-gp will be quantified using a commercially available ELISA(USCN Life Sciences Inc., Wuhan, P.R. China) kit as previously described. Western blot will be used to confirm the ELISA findings. Briefly, 10 µg of total protein and Laemmli loading buffer (2x Laemmli sample buffer, Bio-Rad, Hercules, CA, with β-mercaptoethanol, Sigma, St. Louis, MO) will be denatured at 95˚C for 3 minutes and separated on precast Novex® 4-20% Tris-Glycine Mini Protein Gels (1.0 mm, 9 wells, Life Technologies). Protein will be transferred onto a polyvinylidene fluoride membrane by iBlot® Dry Blotting System (Invitrogen, Carlsbad, CA). The membrane will be blocked and the primary antibody (Monoclonal Anti-P-glycoprotein Clone F4, 1:1000, in 5% milk in TBST) will be added and incubated at 4˚C overnight. After 3 washes of 15 minutes each with TBST, the secondary antibody (Anti-Mouse IgG (Fab specific)–Peroxidase antibody produced in goat in 5% milk in TBST, Sigma) will be incubated for 1 hour at room temperature. The membrane will be washed again 3 times at 15 minutes each with TBST and SuperSignal™ West Pico Chemiluminescent Substrate (Life Technologies) will be applied for 5 minutes before visualization with ChemiDoc MP(Bio-Rad). Recombinant P-gp (USCN Life Sciences Inc., Wuhan, P.R. China) will be used as a control and a monoclonal anti-GAPDH antibody produced in mouse (Sigma Aldrich) will be used as a loading control. Pre and post-treatment Th2 associated cytokines in whole mucus and irrigant efflux including Eotaxin-3, Eosinophilic Cationic Protein(ECP), IL-6, IL-5, and TSLP which have been previously associated with successful CRSwNP treatment response[36][30][3][45] will be tested by commercially available ELISA.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Mucus will be taken from the middle meatus by placing a compressed polyvinyl alcohol sponge (PVA, Medtronic, Minneapolis, MN) within the internal valve for 5 minutes taking care not to abrade the mucosa or contaminate the sponge with blood. The irrigant efflux will be collected in sterile 50mL conical tubes. All samples will be stored in -80°C freezers in the Eaton-Peabody Laboratories(EPL) at MEEI.

7.2.4 SPECIMEN SHIPMENT

N/a

7.3 STUDY SCHEDULE

7.3.1 SCREENING

Phase 1B:

During the screening visit all exclusion/unclusion criteria will be reviewed. If a participant is a good candidate for the study consent will be obtained. Participants will be given an intake questionnaire which queires the presence of a variety of medical parameters which are relevant to the disease process. A pregnancy test will be administered to any female of child bearing age who is sexually active and not using contraception or admits to a possbility of being pregnant. If the participant has used systemic steroids within the past 3 weeks they will be asked to undergo a 3 week washout of steroids before beginning the trial.

Phase 2:

During the screening visit all exclusion/inclusion criteria will be reviewed. If a participant is a good candidate for the study consent will be obtained. Participants will be given an intake questionnaire which queires the presence of a variety of medical parameters which are relevant to the disease process. A pregnancy test will be administered to any female of child bearing age who is sexually active and not using contraception or admits to a pissbility of being pregnant. If the participant has used systemic steroids within the past 3 weeks they will be asked to undergo a 3 week washout of steroids before beginning the trial.

7.3.2 ENROLLMENT/BASELINE

Phase 1B:

Participants will fill out the baseline questionnaires (SNOT-22, VAS). An endoscopy will be recorded and scored. A mucus sample will be collected from the nasal cavity with a small sponge.Participants will be instructed in the proper method for using the verapamil irrigation. They will then be given a baseline EKG and asked to perform the first verapamil irrigation while being monitored for 1 hour by clinical monitoring equipment and study staff. After monitoring the patients will be given a second EKG. The irrigant will be collected. If no EKG abnormalities that are deemed unsafe by the study safety officer are detected during or after the initial irrigation the participants will be prescribed the rest of the verapamil rinse.

Phase 2:

Participants will fill out the baseline questionnaires (SNOT-22, VAS). An endoscopy will be recorded and scored. A mucus sample will be collected from the nasal cavity with a small sponge. Participants will be instructed in the proper method for using the verapamil irrigation. They will then be given a baseline EKG and asked to perform the

first verapamil irrigation while being monitored for 1 hour by clinical monitoring equipment and study staff. After monitoring the patients will be given a second EKG. The irrigant will be collected.. If no EKG abnormalities that are deemed unsafe by the study safety officer are detected during or after the initial irrigation the participants will be prescribed the rest of the verapamil rinse.

7.3.3 FOLLOW-UP

Phase 1B:

Participants will asked to fill out a SNOT-22, VAS, , Satisfaction scale, and Compliance scale. Adherence to the treatment regimen will be evaluated. An endoscopy will be recorded and scored. A mucus sample will be collected from the nasal cavity with a small sponge. Participants will be evaluated for side-effects. An EKG will be taken. If no intermediate toxicity or DLT is experienced the participant will be moved up to the next dose. Participants will be asked to perform the initial irrigation of their next dose while being monitored for 1 hour by clinical monitoring equipment. After monitoring the patients will be given a second EKG. The irrigant will be collected. If no EKG abnormalities that are deemed unsafe by the study safety officer are detected during or after the initial irrigation the participants will be prescribed the rest of the verapamil rinse.

Phase 2:

During the Week 1 follow-up visit participants will asked to fill out a SNOT-22, VAS, , Satifscation scale, and Compliance scale. Adherence to the treatment regimen will be evaluated. Participants will be evaluated for side-effects. An EKG will be taken. An endoscopy will be recorded and scored.

7.3.4 FINAL STUDY VISIT

Phase 1B:

Participants will have their final study visit when they experience DLT and are removed from the study, choose to end their participation, or achive the maximal administered dose. The reason for the drop-out will be noted in the participant file. Participants will be asked to fil out a SNOT-22, VAS, Satisfaction scale, and Compliance scale and will be evaluated for side-effects. An endoscopy will be recorded and scored. A mucus sample will be collected from the nasal cavity with a small sponge. Participants will receive their compensation at the end of the final visit.

Phase 2:

During the Week 4 visit participants will be asked to fill out a SNOT-22, VAS, Satisfaction scale, and Compliance scale. Adherence to the treatment regimen will be evaluated. Participants will be evaluated for side-effects. An endoscopy will be recorded and scored. A mucus sample will be collected from the nasal cavity with a small sponge. Participants will receive their compensation at the end of the final visit.

7.3.5 EARLY TERMINATION VISIT

In the event of an early termination participants will be asked to perform all the procedures for the final visit (Phase 1B and 2) at their final visit.

7.3.7 SCHEDULE OF EVENTS TABLE

	Phase Ib			Phase II			
Procedures	Screening	Enrollment/ Baseline	Weekly/Final Follow-up	Screening	Enrollment/ Baseline	Follow-up (Day 7)	Final Visit (Day 28)
Informed Consent (IC)	x			x			
Intake Form (IF)	x			x			
Pregnancy Test (PT)	x			x			
SNOT-22		x	x		x	x	x
Visual Analog Scale (VAS)		x	x		x	x	x
Electrocardiogram (EKG)		x	x		x	x	
Heart Rate (HR)		x	x		x	x	x
Blood Pressure (BP)		x	x		x	x	x
Lund-Kennedy Score (LKS)		x	x		x	x	x
Nasal irrigation (NI)		x	x		x		x
Mucus sponge (MS)		x	x		x		x
Satisfaction Scale (SS)			x			x	x
Compliance Scale (CS)			x			x	x
Side effects (SE)			x			x	x

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

N/a

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

Participants will be asked to abstain from consuming grapefruit, limes, or pomelos while using the study medication.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

The following drugs will not be permitted for concomitant use with Verapamil HCl: AspirinBeta-blockers, Cimetidine(Tagamet), Clarithromycin(Biaxin), Cyclosporin, Digoxin, Disopyramide(Norpace), Diuretics, Erythromycin, Flecainide, HIV Protease Inhibitors(Indinavir, Nelfinavir, Ritonavir), Quinidine, Lithium, Pioglitazone, Rifampin, and St Johns Wort.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

N/a

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

N/a

7.9 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Participants will not have access to intranasal Verapamil HCl after the trial.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Safety parameters that are also endpoints for Phase Ib are:

- a development of 2nd or 3rd degree heart block as measured by an EKG.
- a heart rate of <50
- an asymptomatic BP reduction >30% from baseline or SBP <90mmHg
- an asymptomatic AMP reduction >30% from baseline or MAP<55
- an asymptomatic DBP reduction >30% from baseline

All safety parameters will be recorded in a partcipants CRF at each study visit. All AEs will require reporting for the protection of human subjects.

If a heart block develops, treatment will be adjusted based on symptomatology. Completely asymptomatic patients can be observed. Patients with mild to moderate symptoms (hypotension, orthostasis, fatigue, dyspnea or angina) should be treated with 1liter NS bolus followed by Calcium Gluconate. Patients who are severely symptomatic and have failed the above interventions can also receive glucagon, norepinephrine, and finally pacing if necessary

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event will be defined as any untoward medical occurrence associated with the use of intranasal Verapamil HCl, whether or not it is considered related to the intervention.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("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); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This study will use the OHRP definition of UP

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 RELATIONSHIP TO STUDY AGENT

The clinician's assessment of an AE's relationship to study agent (drug, biologic, device) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with the study agent, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study agent caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established

8.2.3 EXPECTEDNESS

Dr. Bleier will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Participants will be assessed for AEs during each study visit. All AEs including local and systemic reactions not meeting the criteria for SAEs will be recorded in the CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the participant file throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

All AE's will be reported to both the IRB and DSMB. SAEs will be reported within 24 hours of investigator awareness and non-serious AEs will be reported within 7 days of investigator awareness. The study coordinator will compile a formal report of the event and corrective steps for both the DSMB and IRB. The investigator will be responsible for signing off on AE reports and submitting them to the DSMB and IRB.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

The study clinician will complete a SAE Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the SAE Form and submitted to the DCC/study sponsor within 24 hours of site awareness. See **Section 1, Key Roles** for contact information.
- Other SAEs regardless of relationship, will be submitted to the DCC/study sponsor within 72 hours of site awareness.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the DCC/study sponsor and should be provided as soon as possible. The study sponsor will be responsible for notifying FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB projectnumber;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 7 days of the investigator becoming aware of the problem.

8.4.4 EVENTS OF SPECIAL INTEREST

N/a

8.4.5 REPORTING OF PREGNANCY

Pregnant women will be excluded from this trial. If a participant becomes pregnant during the trial they will be withdrawn and treatment will be immediately halted. The participant will then have safety follow-ups during the course of their pregnancy.

8.5 STUDY HALTING RULES

Administration of study agent will be halted if the trial reaches a 20% AE rate and enrollment screens will stop accepting new study participants. The PI will inform the DSMB members within 24 hours of this occurrence and will provide the DSMB with AE listing reports. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with the study to the PI. The PI will inform the FDA of the temporary halt and the disposition of the study.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise, including Dr. Jeremy Goldfarb, Dr. Christine Finn, and Dr. Hang Lee. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the PI and study staff.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with Good Clinical Practice(GCP), and with applicable regulatory requirements(s). A Data Safety Monitoring Board(DSMB, see section 11) will be established to monitor the study. The DSMB will meet at least twice a year. During Phase Ib the DSMB will monitor the study after the first 3 participants are enrolled and then every 6 patients after that. During Phase II the DSMB will monitor the study after 50% (10 participants) of enrollment has been reached. The DSMB will review all efficacy and safety data and give the PI a report of their findings. The PI will perform internal quality management of study conduct, data collection, documentation, and completion. All EKG and hemodynamic data will be reviewed by a medical internist/ anesthesiologist(JG) who also will act as the chair the DSMB.

The PI will perform internal quality management of study conduct, data collection, documentation and completion.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

All statistical analyses will be performed in conjunction with the consultant biostatistician(Dr. Hang Lee). The proposed sample size of 20 subjects for the Phase II expansion cohort was determined to detect, with an 80% power at a 5% type-1 error rate, a change of 15.9 points on the primary endpoint (ie. SNOT-22 score) between baseline and 4 weeks. This calculation was based on two assumptions. The first is a target effect size of 15.9 which represents a conservative value derived from the established MCID for the SNOT-22 of 8.9[65] and the LSM change in SNOT-22 of 27.7 derived from our previous trial of oral Verapamil(Section R.4.d)[19]. The second is an anticipated standard deviation of 24 which is calculated from the standard deviations of 30 both at the baseline and 8 week time point observed in our oral Verapamil trial and an r2 of 50% between the two time points. With a significance level of 0.05, a standard deviation of 24, and 80% power, a sample size of 20 would be needed to detect a difference of 15.9. Analysis of efficacy will be based on an intention-to-treat population that will include all enrolled patients. A mixed-effect model with repeated measures approach will be used to independently analyze the change in the SNOT-22, VAS, and LKS. An intraclass correlation coefficient (ICC) will be calculated to assess inter-rater agreement with respect to the LKS scores. Descriptive statistics will be used for demographics, baseline characteristics, and safety variables. Linear regression models will be fitted to examine the interaction effect between baseline characteristics, whole mucus and exosomal P-gp concentrations, mucus and irrigant cytokine concentrations, and treatment on change in SNOT-22 while adjusting for the baseline SNOT-22 score. Statistical analyses will be performed using the statistical software SAS (SAS Institute Inc., Cary, NC)

10.2 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoints: SNOT-22
  - H_0: There is no means difference between baseline SNOT-22 scores and 4 week SNOT-22 scores
- Secondary Efficacy Endpoints: VAS and LKS
  - H_0: There is no means difference between baseline VAS scores and 4 week VAS scores
  - H_0: There is no means difference between baseline LKS scores and 4 week LKS scores

10.3 ANALYSIS DATASETS

Phase Ib:

All patients in the Phase Ib arm will be part of the Safety Analysis Dataset

Phase II:

The Phase II participants will be included in an intention-to-treat dataset and a safety analysis dataset.

Participants who used at least 80% of the topical verapamil during the one month period will be considered an evaluable dataset.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

Phase Ib:

Phase Ib will be an intra-pateint, accelerated titration design. The analysis of the safety endpoints will be presented with means and standard deviations of their occurance within the study population.

Phase II:

Phase II will be an open-label trial. Efficacy and safety outcomes will be presented with means and standard deviations.

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Data for the primary efficacy endpoints will be tested to determine if they are parametric. For parametric data the differences between the baseline and subsequent visits will be tested using a Student's t-test. Non-parametric data may be tested using the Mann Whitney U test. Additional statistical methods may be utilized if required. Any deivations from the previously approved statistical plan will be described and justified in a protocol amendment and/or in the final report submitted to this application.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Data for the secondary endpoints will be analyzed using the same methods as the primary endpoints.

10.4.4 SAFETY ANALYSES

The safety of the drug will be determined by analyzing the rate of adverse events in both phases of the trial. An occurance of 10% within the study population will be considered significant.

10.4.5 ADHERENCE AND RETENTION ANALYSES

Adherence to the dosing schedule will be tracked with a dosing calendar that participants will fill out daily. This calendar will be assessed by the study staff at each visit.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Baseline statistics will be calculated using means (standard deviations) and numbers (percentages).

10.4.7 PLANNED INTERIM ANALYSES

10.4.7.1 SAFETY REVIEW

Phase Ib:

This arm will be halted when the MTD has been determined. The proposed safety endpoints will be monitored at each weekly visit. Participants will be formally queried for medication related side effects and will undergo an EKG at each visit, which will be assessed by a medical internist/anesthesiologist.

Phase II:

This arm will be halted if 20% of the population experiences a severe adverse event. This decision will be made at the discretion of the DSMB. Safety endpoints will be monitored at each of the 3 study visits (baseline, week 1, week 4). Participants will be formally queried for medication related side effects and will undergo a repeat EKG at the week 1 visit, which will be assessed by a medical internist/anesthesiologist.

10.4.7.2 EFFICACY REVIEW

No interim analysis will be performed in either arm of this trial.

10.4.8 ADDITIONAL SUB-GROUP ANALYSES

Phase Ib:

The primary safety endpoints will not be sub-divided by demographic characteristics.

Phase II:

The primary endpoint, SNOT22 score, analysis will not be sub-divided by demographic characteristics.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

N/a

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

Individual participant data will be listed be listed by visit number. In Phase Ib visits will be numbered (1,2,3…) by how many visits the participant has before they reach the MTD. In Phase 2 visits will be listed (baseline, week 1, week 4) per the schedule of events.

10.4.11 EXPLORATORY ANALYSES

N/a

10.5 SAMPLE SIZE

The sample size for the Phase II arm was determined in consultation with Dr. Hang Lee (biostatistician). It is based on the MCID for the SNOT-22 and the least suare means value for the SNOT-22 that was determined by the previous oral verapamil trial conducted by Dr. Bleier. The new, combined MCID for the SNOT-22 was determined to be 15.9. The standard deviation for this trial is 24. This was calculated using the standard deviation of the oral verapamil trial (30) and an r^2 of 50%. With a significance level of 0.05, a standard deviation of 24, and 80% power, a sample size of 20 is needed to detect a difference of 15.9.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Participants will be enrolled in the MEEI Sinus Center. Eligible patients will be informed about the trial by their physician in the Sinus Center. If they express interest a member of the study staff will enroll them after passing through all inclusion and exclusion criteria. There will be no blinding or randomization in either phase of this trial.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

N/a

10.6.3 BREAKING THE STUDY BLIND/PARTICIPANT CODE

N/a

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

A Case Report Form (CRF) will be completed for each subject enrolled into the clinical study. The PI and/or their designee will review, approve and sign/date each completed CRF; the PI and/or designee's signature serving as attestation of the PI's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic. Source Data are the clinical findings and observations, test data, and other information contained in Source Documents. Source Documents are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, and recorded data from automated instruments. When applicable, information recorded on the CRF shall match the Source Data recorded on the Source Documents. The EKG findings, SNOT-22, VAS, Side Effects, LKS, satisfaction, and compliance data will be recorded on the CRF which will serve as the primary source data for these data elements. Each form will be recorded in a study chart which is linked to the subject's study ID number. An electronic image capture system will be used to record nasal endoscopy which will be maintained on an encrypted MEEI server.

12 QUALITY ASSURANCE AND QUALITY CONTROL

The PI and DSMB will verify that the study is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g. FDA, Clinicaltrials.gov). The PI and study staff will provide direct access to all source data/documents and reports for the purpose of monitoring and auditing by the DSMB, and inspection by local and regulatory authorities.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The PI will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participatns need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study agent, study procedures, and risks are given to the participants and written documentation of informed consent is required prior to starting administering the study drug. The following materials are submitted with this protocol:

- Informed consent form
- Informed consent script

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The PI and/or designee will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held

in strict confidence. Participants will be linked to their information with a coded identification number. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI.

The DSMB, representatives of the IRB or any other regulatory agency may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES,SPECIMENS OR DATA

Samples and data collected under this protocol may be used to study CRSwNP. No genetic testing will be performed.

Access to stored samples to be limited using a locked freezer at MEEI. Samples and data will be stored using codes assigned by the PI. Data will be kept in password-protected computers. Only the PI and study staff will have access to the samples and data. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained.

13.5 FUTURE USE OF STORED SPECIMENS

Data collected for this study will be analyzed and stored at MEEI. Samples stored for this study will not be used for future research projects.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff under the supervision of the PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

14.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

These documents should be retained for a longer period, however, if required by local regulations. It is the responsibility of the PI to inform the study staff when these documents no longer need to be retained.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

14.4 PUBLICATION AND DATA SHARING POLICY

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as Clinicaltrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee’s responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee
recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.
FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations,of a product subject to FDA regulation;
- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation(other than small feasibility studies) and pediatric postmarket surveillance studies.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

N/a

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the degisn, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts amanged in a way that is appropriateto their participation in the tiral. The study leadership has established policies and procedures for all study group members to disclose oall conclits of interest and will establish a mechanism for the management of all reported dualities of interest.

17 LITERATURE REFERENCES

1. Chin D, Harvey RJ. Nasal polyposis: an inflammatory condition requiring effective anti-inflammatory treatment. Curr. Opin. Otolaryngol. Head Neck Surg. 2013; 21:23–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23172039. Accessed 9 May 2014.
2. Soudry E, Wang J, Vaezeafshar R, Katznelson L, Hwang PH. Safety analysis of long-term budesonide nasal irrigations in patients with chronic rhinosinusitis post endoscopic sinus surgery. Int. Forum Allergy Rhinol. 2016; 6:568–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26879335.
3. Bachert C, Mannent L, Naclerio RM, et al. Effect of Subcutaneous Dupilumab on Nasal Polyp Burden in Patients With Chronic Sinusitis and Nasal Polyposis: A Randomized Clinical Trial. JAMA 2016; 315:469–79. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26836729.
4. Lane AP, Truong-Tran QA, Schleimer RP. Altered expression of genes associated with innate immunity and inflammation in recalcitrant rhinosinusitis with polyps. Am. J. Rhinol. 2013; 20:138–44. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2810150&tool=pmcentrez&rendertype=abstract. Accessed 20 August 2014.
5. Van Zele T, Holtappels G, Gevaert P, Bachert C. Differences in initial immunoprofiles between recurrent and nonrecurrent chronic rhinosinusitis with nasal polyps. Am. J. Rhinol. Allergy 28:192–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24980230. Accessed 16 December 2014.
6. Derycke L, Eyerich S, Van Crombruggen K, et al. Mixed T helper cell signatures in chronic rhinosinusitis with and without polyps. PLoS One 2014; 9:e97581. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4049589&tool=pmcentrez&rendertype=abstract. Accessed 16 December 2014.
7. Nagarkar DR, Poposki JA, Tan BK, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. J. Allergy Clin. Immunol. 2013; 132:593–600.e12. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3759596&tool=pmcentrez&rendertype=abstract. Accessed 16 December 2014.
8. Bleier BS, Kocharyan A, Singleton A, Han X. Verapamil modulates interleukin-5 and interleukin-6 secretion in organotypic human sinonasal polyp explants. Int. Forum Allergy Rhinol. 2014; 5:10–13. Available at: http://doi.wiley.com/10.1002/alr.21436. Accessed 19 December 2014.

9. Sachse F, Becker K, von Eiff C, Metze D, Rudack C. Staphylococcus aureus invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells in vitro. Allergy 2010; 65:1430–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20456313. Accessed 9 May 2014.
10. Bleier BS, Article O. Regional expression of epithelial MDR1/P-glycoprotein in chronic rhinosinusitis with and without nasal polyposis. Int. Forum Allergy Rhinol. 2012; 2:122–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22223515. Accessed 21 January 2014.
11. Feldman RE, Lam AC, Sadow PM, Bleier BS. P-glycoprotein is a marker of tissue eosinophilia and radiographic inflammation in chronic rhinosinusitis without nasal polyps. Int. Forum Allergy Rhinol. 2013; 3:684–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23677641. Accessed 11 February 2014.
12. Bleier BS, Nocera AL, Iqbal H, et al. P-glycoprotein promotes epithelial T helper 2-associated cytokine secretion in chronic sinusitis with nasal polyps. Int. Forum Allergy Rhinol. 2014; 4:488–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24599606. Accessed 9 May 2014.
13. Nocera AL, Meurer AT, Miyake MM, Sadow PM, Han X, Bleier BS. Secreted P-glycoprotein is a noninvasive biomarker of chronic rhinosinusitis. Laryngoscope 2016; Available at: http://www.ncbi.nlm.nih.gov/pubmed/27577924.
14. Tsuruo T, Iida H, Yamashiro M, Tsukagoshi S, Sakurai Y. Enhancement of vincristine- and adriamycin-induced cytotoxicity by verapamil in P388 leukemia and its sublines resistant to vincristine and adriamycin. Biochem. Pharmacol. 1982; 31:3138–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7150340.
15. Hashioka S, Klegeris A, McGeer PL. Inhibition of human astrocyte and microglia neurotoxicity by calcium channel blockers. Neuropharmacology 2012; 63:685–691.
16. Li G, Qi XP, Wu XY, et al. Verapamil modulates LPS-induced cytokine production via inhibition of NF-kappa B activation in the liver. Inflamm. Res. 2006; 55:108–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16673153. Accessed 30 April 2014.
17. Matsumori A, Nishio R, Nose Y. Calcium Channel Blockers Differentially Modulate Cytokine Production by Peripheral Blood Mononuclear Cells. Circ. J. 2010; 74:567–571. Available at: http://joi.jlc.jst.go.jp/JST.JSTAGE/circj/CJ-09-0467?from=CrossRef. Accessed 30 April 2014.
18. Khakzad MR, Mirsadraee M, Mohammadpour A, et al. Effect of verapamil on bronchial goblet cells of asthma: an experimental study on sensitized animals. Pulm. Pharmacol. Ther. 2012; 25:163–168.
19. Miyake MM, Nocera AL, Levesque P, et al. Double-Blind Placebo-Controlled Randomized Clinical Trial of Verapamil for Chronic Rhinosinusitis with Nasal Polyps. J. Allergy Clin. Immunol. (in press)
20. Smith SBSS, Evans CT, Tan BK, Chandra RK, Smith SBSS, Kern RC. National burden of antibiotic use for adult rhinosinusitis. J. Allergy Clin. Immunol. 2013; 132:1230–1232.
21. Hsiao C-J, Cherry DK, Beatty PC, Rechtsteiner E a. National Ambulatory Medical Care Survey: 2007 summary. Natl. Health Stat. Report. 2010; :1–32.
22. Wallace D V., Dykewicz MS, Bernstein DI, et al. The diagnosis and management of rhinitis: An updated practice parameter. J. Allergy Clin. Immunol. 2008; 122.
23. Smith K a, Orlandi RR, Rudmik L. Cost of adult chronic rhinosinusitis: A systematic review. Laryngoscope 2015; :1–10.
24. Rudmik L, Smith TL, Schlosser RJ, Hwang PH, Mace JC, Soler ZM. Productivity costs in patients with refractory chronic rhinosinusitis. Laryngoscope 2014; 124:2007–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24619604.
25. Hedman J, Kaprio J, Poussa T, Nieminen MM. Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. Int. J. Epidemiol. 1999; 28:717–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10480701.
26. Klossek JM, Neukirch F, Pribil C, et al. Prevalence of nasal polyposis in France: a cross-sectional, case-control study. Allergy 2005; 60:233–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15647046.
27. Johansson L, Akerlund A, Holmberg K, Melén I, Bende M. Prevalence of nasal polyps in adults: the Skövde population-based study. Ann. Otol. Rhinol. Laryngol. 2003; 112:625–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12903683.
28. Vaidyanathan S, Barnes M, Williamson P, Hopkinson P, Donnan PT, Lipworth B. Treatment of chronic rhinosinusitis with nasal polyposis with oral steroids followed by topical steroids: a randomized trial. Ann. Intern. Med. 2011; 154:293–302. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21357906.

29. Rupa V, Jacob M, Mathews MS, Seshadri MS. A prospective, randomised, placebo-controlled trial of postoperative oral steroid in allergic fungal sinusitis. Eur. Arch. Otorhinolaryngol. 2010; 267:233–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19714349.
30. Van Zele T, Gevaert P, Holtappels G, et al. Oral steroids and doxycycline: two different approaches to treat nasal polyps. J. Allergy Clin. Immunol. 2010; 125:1069–1076.e4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20451040.
31. Hissaria P, Smith W, Wormald PJ, et al. Short course of systemic corticosteroids in sinonasal polyposis: a double-blind, randomized, placebo-controlled trial with evaluation of outcome measures. J. Allergy Clin. Immunol. 2006; 118:128–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16815148.
32. Rotenberg BW, Zhang I, Arra I, Payton KB. Postoperative care for Samter's triad patients undergoing endoscopic sinus surgery: a double-blinded, randomized controlled trial. Laryngoscope 2011; 121:2702–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21997904.
33. Rawal RB, Deal AM, Ebert CS, et al. Post-operative budesonide irrigations for patients with polyposis: a blinded, randomized controlled trial. Rhinology 2015; 53:227–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26363164.
34. Mjösberg JM, Trifari S, Crellin NK, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat. Immunol. 2011; 12:1055–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21909091. Accessed 30 April 2014.
35. Peters AT, Kato A, Zhang N, et al. Evidence for altered activity of the IL-6 pathway in chronic rhinosinusitis with nasal polyps. J. Allergy Clin. Immunol. 2010; 125:397–403.e10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2828355&tool=pmcentrez&rendertype=abstract. Accessed 9 May 2014.
36. Gevaert P, Lang-Loidolt D, Lackner A, et al. Nasal IL-5 levels determine the response to anti-IL-5 treatment in patients with nasal polyps. J. Allergy Clin. Immunol. 2006; 118:1133–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17088140.
37. Gevaert P, Van Bruaene N, Cattaert T, et al. Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. J. Allergy Clin. Immunol. 2011; 128:989-95–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21958585.
38. Wenzel S, Ford L, Pearlman D, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N. Engl. J. Med. 2013; 368:2455–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23688323.
39. Tomassen P, Vandeplas G, Van Zele T, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. J. Allergy Clin. Immunol. 2016; Available at: http://www.ncbi.nlm.nih.gov/pubmed/26949058.
40. Lässer C, Neil SEO, Shelke G V, et al. Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation. J. Transl. Med. 2016; :1–14. Available at: "http://dx.doi.org/10.1186/s12967-016-0927-4.
41. Lee TH, D'Asti E, Magnus N, Al-Nedawi K, Meehan B, Rak J. Microvesicles as mediators of intercellular communication in cancer-the emerging science of cellular 'debris'. Semin. Immunopathol. 2011; :1–13.
42. Lee Y, El Andaloussi S, Wood MJ a. Exosomes and microvesicles: Extracellular vesicles for genetic information transfer and gene therapy. Hum. Mol. Genet. 2012; 21:125–134.
43. Mack M, Kleinschmidt a, Brühl H, et al. Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. Nat. Med. 2000; 6:769–775.
44. Bleier BS, Nocera AL, Iqbal H, et al. P-glycoprotein promotes epithelial T helper 2-associated cytokine secretion in chronic sinusitis with nasal polyps. Int. Forum Allergy Rhinol. 2014; Available at: http://www.ncbi.nlm.nih.gov/pubmed/24599606. Accessed 9 May 2014.
45. Bleier BS, Singleton A, Nocera AL, Kocharyan A, Petkova V, Han X. P-glycoprotein regulates Staphylococcus aureus enterotoxin B–stimulated interleukin-5 and thymic stromal lymphopoietin secretion in organotypic mucosal explants. Int Forum Allergy Rhinol. 2015; In press.:1–9.
46. Peric A, Vojvodic D, Matkovic-Jozin S. Effect of long-term, low-dose clarithromycin on T helper 2 cytokines, eosinophilic cationic protein and the 'regulated on activation, normal T cell expressed and secreted' chemokine in the nasal secretions of patients with nasal polyposis. J. Laryngol. Otol. 2012; 126:495–502.

47. Hashemi M, Fereidani A, Berjis N, Okhovat SAR, Eshaghain A. Effectiveness of itraconazole on clinical symptoms and radiologic findings in patients with recurrent chronic rhinosinusitis and nasal polyposis. Adv. Biomed. Res. 2014; 3:162.
48. Kocharyan A, Feldman R, Singleton A, Han X, Bleier BS. P-glycoprotein inhibition promotes prednisone retention in human sinonasal polyp explants. Int. Forum Allergy Rhinol. 2014; 4:734–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25145685.
49. Watling S, Engelhardt J, Kandrotas R, et al. Comparison of intranasal versus intravenous verapamil bioavailability. Int. J. Clin. Pharmacol. Ther. Toxicol. 1993; 31:100–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8458675.
50. Nocera AL, Muerer AT, Singleton A, et al. Intact Soluble P-glycoprotein is Secreted by Sinonasal Epithelial Cells. Am. J. Rhinol. Allergy 2016;
51. Becker WJ. Cluster headache: conventional pharmacological management. Headache 53:1191–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23773141. Accessed 22 September 2014.
52. Abdel Mouez M, Zaki NM, Mansour S, Geneidi AS. Bioavailability enhancement of verapamil HCl via intranasal chitosan microspheres. Eur. J. Pharm. Sci. 2014; 51:59–66.
53. Arnold TH, Tackett RL, Vallner JJ. Pharmacodynamics of acute intranasal administration of verapamil: comparison with i.v. and oral administration. Biopharm. Drug Dispos. 1985; 6:447–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4084670.
54. Vogelgesang B, Echizen H, Schmidt E, Eichelbaum M. Stereoselective first-pass metabolism of highly cleared drugs: studies of the bioavailability of L- and D-verapamil examined with a stable isotope technique. Br. J. Clin. Pharmacol. 1984; 18:733–740.
55. Gehanno P, Darantiere S, Dubreuil C, et al. A prospective, multicentre study of moxifloxacin concentrations in the sinus mucosa tissue of patients undergoing elective surgery of the sinus. J. Antimicrob. Chemother. 2002; 49:821–826.
56. Dinis PB, Monteiro MC, Silva N, Martins ML, Morais JG. Sinus tissue concentration of moxifloxacin after a single oral dose. Ann. Otol. Rhinol. Laryngol. 2004; 113:142–146.
57. Edmiston CE, Krepel CJ, Seabrook GR, et al. Tissue and fluid penetration of garenoxacin in surgical patients. Surg. Infect. (Larchmt). 2007; 8:179–88. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17437363.
58. Sarangapani R, Teeguarden JG, Gentry PR, Clewell HJ, Barton H a, Bogdanffy MS. Interspecies dose extrapolation for inhaled dimethyl sulfate: a PBPK model-based analysis using nasal cavity N7-methylguanine adducts. Inhal. Toxicol. 2004; 16:593–605. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16036752.
59. Hopkins C, Slack R, Lund V, Brown P, Copley L, Browne J. Long-term outcomes from the english national comparative audit of surgery for nasal polyposis and chronic rhinosinusitis. Laryngoscope 2009; 119:2459–2465.
60. Meltzer EO, Hadley J, Blaiss M, et al. Development of questionnaires to measure patient preferences for intranasal corticosteroids in patients with allergic rhinitis. Otolaryngol. - Head Neck Surg. 2005; 132:197–207.
61. Lund VJ, Kennedy DW. Staging for rhinosinusitis. Otolaryngol. Head. Neck Surg. 1997; 117:S35-40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9334786.
62. Orlandi RR, Kingdom TT, Hwang PH, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. Int. Forum Allergy Rhinol. 2016; 6 Suppl 1:S22–S209. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26889651.
63. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr. Protoc. Cell Biol. 2006; Chapter 3:Unit 3.22.
64. Mallegol J, Van Niel G, Lebreton C, et al. T84-Intestinal Epithelial Exosomes Bear MHC Class II/Peptide Complexes Potentiating Antigen Presentation by Dendritic Cells. Gastroenterology 2007; 132:1866–1876.
65. Hopkins C, Gillett S, Slack R. Psychometric validity of the 22-item Sinonasal Outcome Test. Clin. … 2009; :447–454.

APPENDIX

Version	Date	Significant Revisions