Effects of Broccoli Sprout Extract on Allergic Rhinitis

NCT02885025

Study Protocol and Statistical Analysis Plan

November 2, 2018

RESEARCH PLAN

BACKGROUND AND SIGNIFICANCE

Safe alternative nutritional supplementation is needed for atopic disorders suffered by US veterans:

Allergic Rhinitis a major condition impairing nasal health, effects 10-30% worldwide and 8% nationwide (1). A 10 year retrospective review revealed the majority of patients receiving care at the VA Greater Los Angeles Healthcare System (VAGLAHS) suffered from Allergic Rhinitis (2). From suffering comes cost, both indirect and direct. This can manifest as lost days of work and medication expense. Among atopic disorders, completed surveys determined allergic rhinitis as the highest cost in regards to both presenteeism and absenteeism, with the average total productivity loss per employee surveyed at 593 dollars per year (3). Over 3.4 billion dollars is spent directly treating allergic rhinitis, with 46.6% attributed to prescription medications. Despite no cost effective treatment for allergic rhinitis, nasal corticosteroids appear to be the most beneficial. Patients suffering from allergic rhinitis seek alternative therapies since 2/3rds report daily impairment from rhinitis symptoms despite the use of available medications (4).

The Association between Pollutants and Allergy:

The increase prevalence in allergic disorders may be attributable to pollutant factors (5). Bench Research led to a valid explanation of how pollutants can act as an adjuvant to allergens in susceptible patients. Original work discovered pollutant diesel exhaust particles (DEP) increase reactive oxygen species (ROS) in respiratory epithelium cells. This overabundance of oxidative stress leads increases intracellular nuclear factor kappa beta (NfKB) and Mitogen Activated Protein Kinase (MAP-kinase) cascades causing atopic cytokine production (6-9).

Animal Models investigating the association between Pollutants and Allergy:

Mice models validated the association between pollutant exposure and allergic response increase. Intranasal administration of diesel exhaust particles (DEP) caused an enhanced IgE response (10). More specifically administering DEP to dust mite sensitized mice enhanced the

production of dust mite specific IgE (11). Further experiments on mice solidified the concept that pollutants cause an increase allergic response when mice given DEP plus allergen were later found to have increased levels of atopic cytokines IL4 and IL5 within lungs and lymph nodes (12, 13).

Human Models investigating the association between Pollutants and Allergy:
These findings were later replicated in human studies. Purified human B cells cultured with TH2 cytokines and DEP significantly increased IgE production when compared to B cells cultured with cytokines alone (14). Human studies demonstrated instillation of DEP through nasal lavage triggered an increase in nasal mucosa IgE production. (15). Nasal instillation of both DEP and ragweed produced increased atopic cytokines IL4, 5,6,10 and 13 and ragweed IgE 50 fold (16). Further review of these studies noted this increase in allergic biomarkers varied among individuals, with some having a drastic response and others no response (17).

Phase II enzymes and Allergic Rhinitis:
The exaggerated atopic response from DEP when present with allergen exposure can be prevented by antioxidant production. Genetic polymorphisms in phase 2 antioxidant enzymes among individuals can determine how pollutants influence atopy. Endogenous anti-oxidant phase 2 enzymes GSTT1, GSTM1 and GSTP1 play a vital role in preventing the production of cytokines through restoration of redox balance between reduced and oxidized glutathione. Null genotypes for GSST1 and GSTM1 are more prevalent in atopic subjects (18), which could explain their diminished ability to produce phase II antioxidants leading to unchecked balance of oxidation. When exposed to pollutants, patients having null mutations in GST increase production of IgE and histamine levels respectively (19, 20). Patients with GST null mutations suffering from allergic rhinitis may respond best to antioxidant therapy.

The use of antioxidants in treating Allergic Conditions:
Success rates in treating atopic conditions with antioxidants have been mixed (21-23). The importance of evaluating the patient for GST polymorphisms is supported by studies showing patients with GSTM1 null genotypes were more likely to have a greater detrimental loss of pulmonary function with exposure to ozone and a more positive response to consumption of Vitamin C/Vitamin E antioxidants supplementation (24, 25).

Sulforaphane for treating allergic conditions:
The antioxidant Sulforaphane increases phase II enzyme production through activation of the Nrf2 transcription factor and the anti-oxidant response element (ARE) leading to an increase production of phase II enzymes (26-30). Sulforaphane also interferes with the pro-inflammatory pathways NkKB and AP1 responsible for the production of TH2 cytokines and IgE.

Sulforaphane has been shown to block B cell IgE production (31) and to inhibit IL8 production in human bronchial airway epithelial cell lines after exposure to DEP via inducing nuclear factor like 2, leading to enhanced Phase II enzyme production (32). A recent study showed that ova sensitized mice treated with SFN had a diminished inflammatory response after ova challenge leading to diminished hyper responsive airways (33).

Broccoli Sprout for improving nasal health:
Broccoli sprouts (BSE), members of the cruciferous vegetable family, have extremely high concentrations of sulforaphane (26). The first placebo controlled BSE human study performed at our partnering institution involved administering escalating doses of broccoli sprout homogenate (BSH) to subjects. Phase II enzymes were shown to increase proportionally to the dose of BSE

given. Furthermore the induction of individual Phase II enzymes was inversely correlated with the expression of phase II enzymes (glutathione-s-transferase M1, glutathione-s-transferase P1, NADPH quinone oxidoreductase, and hemoxygenase-1) as revealed by genetic testing. Patients with GSTM1 null or GSTP1 Ile/Ile105 polymorphisms known to endogenously produce the lowest Phase II enzyme levels responded best to BSE (34).

PRELIMINARY STUDIES:

Administering BSE to allergic subjects was first performed in a recent NIH sponsored study by Dr. Li and colleagues at the University of California, Los Angeles, a partnering institution to the VAGLAHS. Subjects with cat allergy through skin testing diagnosed allergic rhinitis were challenged with 300µg DEP (equivalent to daily PM exposure levels on LA freeway) then underwent 4 weeks of washout, followed by daily BSE administered as a single oral morning dose (11umol sulforaphane equivalent to that formed from consuming 100 to 200 grams of broccoli) for four days. The diesel exhaust particle challenge was repeated after BSE consumption. The average nasal white blood cell counts increased by at least 4 fold ($p<0.0005$) within 24 hours post-DEP exposure and there was significant attenuation of cell count post-DEP exposure when patients were given daily BSE for four days prior to DEP challenge ($p<0.01$ at 6 hours and $p<0.0001$ at 24 hours following DEP challenge). There was no correlation between the inflammatory response and the presence of GSTM1 null orGSTP1 Ile/Ile105 polymorphisms (35). Failure to determine a statistically significant difference in response among the genetic polymorphisms may have been due to the low subject number.

We are proposing a double blinded randomized placebo controlled study to investigate the effectiveness of BSE on nasal health among US veterans with a diagnoses seasonal grass allergic rhinitis in an urban environment where DEP levels are high.

During the study, GST genotype data will be collected in order to obtain data for future exploratory analysis.

RESEARCH DESIGN AND METHODS

Objectives:
Primary Objective:
To conduct a double-blind, randomized, placebo-controlled trial to compare clinical measurements for allergic rhinitis following *Timothy, Bermuda or Johnson* grass allergen (phleum pretense*, cynodon dactylon, or songhum halepense*) challenge before and after initiation of BSE supplementation. Clinical measurements will be derived from the total nasal symptoms score (TNSS) and peak nasal inspiratory flow (PNIF).

Secondary Objectives:
To conduct a randomized controlled trial to compare biomarker measurements for allergic rhinitis following grass allergen challenge before and after initiation of BSE supplementation. Biomarkers will include the following inflammatory cytokines: Interleukin 4, Interleukin 5, Interleukin 13 along with Tryptase and Eosinophil cationic protein (ECP) obtained through polyurethane sponges placed into both nostrils.

To conduct an exploratory genetic analysis of the 3 glutathione S-transferase (GST) genes (GSTM1, GSTT1, and GSTP1) for future investigation into how genetic polymorphisms effect the response to BSE supplementation in atopic disorders.

Overview of Study Design
The proposed study is a double-blind, randomized, placebo-controlled trial consisting of four arms. A total of *190* subjects diagnosed with grass (p. pretense*, c. cynodon, and/or s songhum*) specific allergic rhinitis will be randomly assigned to one of four groups:
1. nasal corticosteroid + Placebo tablet
2. placebo nasal spray + Placebo tablet
3. nasal corticosteroid + Avmacol®
4. placebo nasal spray + Avmacol®

Avmacol is a supplement that supports sulforaphane production by providing glucoraphanin and an active myrosinase enzyme via our Sulforaphane Production System. Subjects will be given 4 tablets to take with their evening meal.

Following consent, a list of antioxidant foods (including sulforaphane containing foods) and prohibited medications to avoid will be given to all subjects then subjects will be scheduled for visit 1 within <20 days.

At visit 1, Subjects will undergo epicutaneous hypersensitivity skin testing to grass allergens prevalent in southern California: Johnson Grass (Sorghum Halepense), Bermuda Grass (Cynodon Dactylon), and Timothy Grass (Phleum Pretense), along with dog, cat and perennial allergens: dust mite (dermatophagoides pteronyssinus, dermatophagoides farina), and common mold allergens (alternaria, aspergillus, cladosporium, and penicillium) (36). Subjects with positive skin test results to one or more grass allergens and either negative skin test to perennial allergens (dust mite or mold) or positive tests to perennial allergens (dust mite or mold) without a correlating clinical history consistent with perennial allergic rhinitis will undergo grass allergen nasal provocation testing. Subjects with a positive skin test to cat or dog and clinical symptoms consistent with cat or dog triggered allergic rhinitis will be asked to avoid cats and or dogs for the entire study. For subjects who have an epicutaneous allergen skin or blood test on file completed by the VA Allergy Clinic within ≤1 year that confirms a positive to grass pollens the results will be used to proceed onto the up-dosing nasal challenge. Subjects experiencing a positive nasal provocation test defined by a total nasal symptom score (TNSS) over 7, or PNIF is reduced by ≥ 50% from baseline redaing will be tested for serum GSTM1 polymorphism and baseline sulforaphane levels and will proceed to visit 2. Subjects failing to mount a positive response with the highest concentration (10,000 BU/cc for p. phleum and c. cynodon, or 1:100 w/v for s. sorghum) will be withdrawn from the study..

Nasal challenge will repeat at visit 2, two weeks after visit 1, and visit 3, 3 weeks after beginning intervention at visit 2. This challenge was developed and perfected by Guy W. Scadding PhD and colleagues and showed reproducibility in a 2012 study (36). Prior to and following each challenge, subjects will undergo spirometry. Challenges will not be performed in patients with forced expiratory volume in 1 second (FEV1) values less than 70%. The visit 1 nasal challenge will be considered a graded up-dosing challenge needed to determine the dose used at visits 2 and 3.

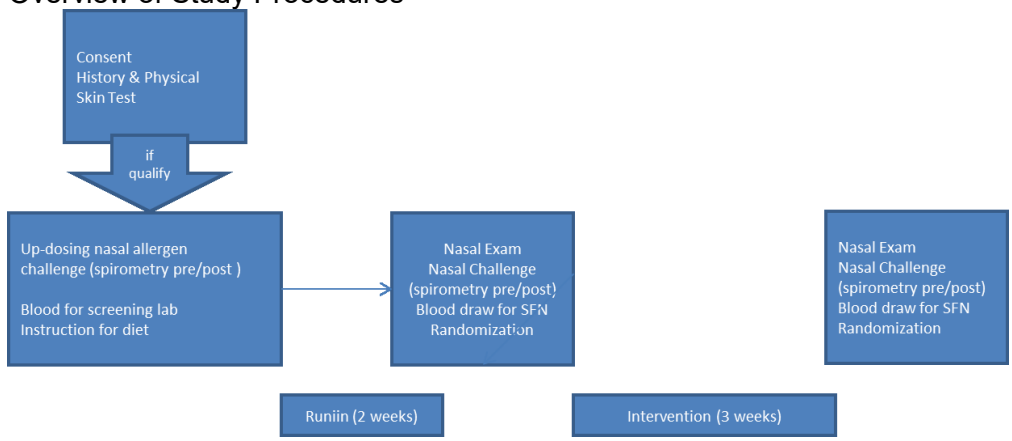
At visit 2 *(2* weeks following visit 1) and visit 3 (5 weeks following visit 1), subjects will undergo a single nasal allergen challenge with the effective dose determined during the up-dosing challenge at visit 1. Total nasal symptom score and peak nasal inspiratory flow will be recorded throughout all nasal challenge procedures.

Biomarkers collected through sponges placed in both nasal passages during the challenge will include IL4, IL5, IL13, eosinophil cationic protein and tryptase. At the end of visit 2, patients will be randomized and will be instructed to begin the following evening, to return in 3 weeks for another nasal challenge.

Throughout the study, serum sulforaphane levels for each subject will be checked at visits 1, 2 and 3 to monitor adherence.

All study procedures will be conducted at the VAGLAHS. Subjects will be assigned an appointment day and appointment time for each scheduled visit. All appointments will be at approximately the same time of the day for all subjects to avoid diurnal variation.

Figure 1. Overview of Study Procedures



Study Population

General Considerations:

Subjects participating in the study are followed at two VA medical centers and one academic medical center. All subjects are non-smoking male or female US veterans aged 18 years and older with symptoms consistent with seasonal summer allergic rhinitis (rhinorrhea, nasal congestion, postnasal drainage sneezing, and/or nasal or eye pruritus). There will be no exclusion based on gender or ethnic/racial background.

Inclusion Criteria

1. Females and males 18 years or older.
2. History consistent with seasonal allergic rhinitis consistent with grass allergy (symptoms during summer months, June through August, for at least two consecutive seasons).
3. Not currently taking any medications for allergic rhinitis.
4. Provide written informed consent.
5. Willing and able to comply with all aspects of the protocol.

Exclusion Criteria

1. The subject has any uncontrolled or serious disease, or any medical or surgical or condition that in the opinion of the investigator(s) could affect the subject's safety and/or interfere with the study assessments.
2. History of anaphylaxis to environmental allergens.or an unknown trigger.
3. History of broccoli allergy.
4. Recent upper respiratory infection (less than 4 weeks prior to study) or other active Infection.

5. Active smoker.
6. Currently receiving allergy immunotherapy.
7. History of rhinitis exacerbation within the past 2 weeks.
8. Use of non-selective Beta-Blocker.
9. Inability to give written informed consent.
10. History or evidence of non-stable cognitive capacity within less than 1 year (i.e. Alzheimer's disease, dementia, bipolar disorder) that in the opinion of the investigator(s) could affect the subject's safety and/or interfere with the study assessments.
11. Pregnancy.
12. Perennial rhinitis.
13. Uncontrolled asthma.
14. FEV1 (forced expiratory volume in 1 s) <70% predicted at screening.

Recruitment:

In order for 152 subjects to complete the study, 475 subjects will be recruited for enrollment under the assumption 50% (237) of subjects will have seasonal allergic rhinitis to grass confirmed on skin testing, 80% (190) of subjects will have a positive nasal challenge and 80% (152) of subjects will complete the study.

Subject recruitment will occur at three facilities: VA Greater Los Angeles Health Care System (VA GLA) , VA Long Beach Health Care System (VA LB) and the University of California Los Angeles (UCLA) Medical Center. Within each facility, recruitment will occur at primary and specialty (allergy) clinics.

Recruitment at VA Greater Los Angeles and VA Long Beach Health Care Centers:

Primary Care Clinic Recruitment:

Investigators will work together with providers assigned to Patient Aligned Care Teams (PACT). On average, PACTs follow 44,000 patients at VA Long Beach and 63,000 at VA Greater Los Angeles. Assuming involvement with primary care providers following 10,000 subjects at VA GLA and 10,000 subjects at VA LB, At each facility, 2,000 (20%) will should have a diagnoses of allergic rhinitis. 600 (30%) will experience allergic rhinitis symptoms during the summer months and 300 (50%) of these subjects will have symptoms limited to summer months. We estimate at total of 600 potential recruitment subjects from primary care clinics at both VA GLA and VA LB.

Allergy Clinic Recruitment:

Within the past year, 40% (320) of new consultations at VA Greater Los Angeles Healthcare System and 68% (208) of new consultations at VA Long Beach Healthcare System were requested to evaluate allergic rhinitis. Based on these figures, we would anticipate a total of 30% having allergic rhinitis associated with summer months and then 50% of these patients having allergic rhinitis limited to summer months or 79 (48 + 31) potential subjects at VA GLA and VA LB combined.

Recruitment at the University of California Los Angeles Medical Center:

In Southern California, patients are seen at 16 satellite primary care clinics affiliated with UCLA Medical Center. Adult Allergists practice at four of these locations (Porter Ranch CA, Santa Monica CA, Thousand Oaks CA, Torrance CA.) Investigators will work with providers at UCLA affiliated clinics in identifying potential subjects. (see Contingency Plan)

Use of Flyers at Institutions:

In addition to the above recruitment strategy, flyers will be placed in primary care, ear nose and throat, and dermatology clinics at VAGLAHS and VALBHCS with contact information for enrollment. Potential subjects who call will be preliminary screened via phone call screening script to determine possible eligibility. Those who pass the preliminary screening process will have an appointment scheduled.

Recruitment from VA GLA Pharmacy Database:
In addition, we will work with pharmacy to retrieve patients prescribed nasal corticosteroids limited to summer months. These patients have a higher likelihood of suffering from grass pollen allergic rhinitis.

Contingency Plan:
Participation recruitment will be followed closely. If needed, we will submit a request for expansion to involve non veterans. The VA GLA is close to UCLA. Students/staff/potential subjects attending UCLA may qualify for inclusion into the study.

Medical History:
A medical history will be obtained to confirm whether patient has clinical symptoms consistent with summer seasonal rhinitis (during grass season) allergic rhinitis.

Procedures at Each Visit:

Consenting (<20 days to Visit 1)
-Affirm eligibility, check medication use.
-Review study with patient and if subject agrees to enroll, obtain subjects written informedconsent.
-Instructions for avoidance of sulforaphane containing food products in diet.
- Review concomitant medication use.
-Instructions for avoidance of the following medications: oral antihistamines, nasal corticosteroids, nasal mast cell stabilizers, nasal antihistamines, leukotriene antagonists.
-Proceed onto visit 1, or schedule subject for visit 1 ≤20 days from providing written consent.

Visit 1 (Day 1)

-Spirometry performed (Note: subjects' have up to 3 attempts to FEV1 of ≥70% to continue on with this study).
-Collect blood pressure, heart rate, height and weight.
- Check concomitant medication use since last visit.
-General physical examination.
-Nasal examination.
-Epicutaneous Allergen Skin Testing. Patients who qualify based on skin test results will proceed with up-dosing nasal challenge to the positive skin test grass allergen. Subjects that have completed epicutaneous allergen skin or blood test at the VA Allergy Service within ≤ 1 year with positive results to grass pollens will to proceed onto the up-dosing nasal challenge using the results on file.
-Updosing nasal challenge. Patients with positive nasal challenge will continue as follows:
-Obtain blood work to determine sulforaphane baseline level and genotype for GSTM1 polymorphisms.
- Schedule subject for visit 2.

Visit 2 (Day 14)
-Check concomitant medication use since last visit.
-Collect blood pressure, heart rate and weight.
-Spirometry performed
-General physical examination.
-Nasal examination.
-nasal lavage
-Obtain blood work for sulforaphane level check.
-Grass Nasal Challenge.
-Randomize patients into one of four groups.
-Medication Dispensing (to begin evening of Day 15).

Visit 3 (Day *35)*
-Check concomitant medication use since last visit.
- Collect medication dispensed at last visit (research team complete medication compliance).
-Collect blood pressure, heart rate and weight.
-Spirometry performed
-General physical examination.
-Nasal examination.
-Nasal lavage
-Obtain blood work for sulforaphane level check.
-Grass nasal Challenge.

Study Schedule:

The typical schedule is shown as an example for consenting.

Consenting (<20 days prior to visit 1) Activitity (in sequence):

Affirm eligibility, check medication use.

Review study with patient and if subject agrees to enroll, obtain subjects written informedconsent.

Instructions for avoidance of sulforaphane containing food products in diet prior to visit 1 and throughout the study.

Instructions for avoidance of the following medications: oral antihistamines, nasal corticosteroids, nasal mast cell stabilizers, nasal antihistamines, leukotriene antagonists prior to visit 1 and throughout the study.

Proceed onto visit 1, or schedule subject for visit 1 within ≤ 20 days from providing written consent.

The typical schedule is shown as an example for the enrollment visit (Visit 1).

Visit 1 (Day 1) Activity (in sequence):

Subject arrives at the Allergy/Immunology Clinic.

Spirometry will be performed before skin test. (Note: subjects' have up to 3 attempts to FEV1 of ≥70% to continue on with this study.)

Vital signs will be collected (heart rate, blood pressure, weight, height) and general physical examination along with nasal examination.

Subjects undergo epicutaneous skin testing. (Subjects who qualify based on skin test will continue with nasal challenge). For subjects who have an epicutaneous allergen skin or blood test on file completed by the VA Allergy Clinic within ≤ 1 year that confirms a positive to grass pollens the results will be used to proceed onto the up-dosing nasal challenge.

Up-dosing nasal allergen challenge will be performed

Subjects testing positive to nasal challenge will proceed with blood work to check sulforaphane Baseline levels and genotype for GSTM1 polymorphisms

The typical schedule is shown as an example for a follow up evaluation day for visit 2.

Visit 2 Activity (in sequence):

Subject arrives at the Allergy/Immunology Clinic.

Vital signs (heart rate, blood pressure and weight) , *nasal examination* , concomitant medication and diet checked since last visit.

Spirometry will be performed. (Note: subjects' have up to 3 attempts to FEV1 of ≥70% to continue on with this study.)

general physical examination along with nasal examination.

Nasal lavage performed.

Blood work obtained for sulforaphane level

Nasal allergen Challenge followed by monitoring explained below

Randomization occurs. Instructions for avoidance of sulforaphane containing food products in diet and for avoidance of medications reiterated.

The typical schedule is shown as an example for a follow up evaluation day for visit 3.

Visit 3 Activity (in sequence):

Subject arrives at the Allergy/Immunology Clinic.

Study medication disepensed at visit 2 are returned by the subject and a compliance check is performed the study team.

Vital signs (heart rate, blood pressure and weight), *nasal examination*, concomitant medication and diet checked since last visit.

Spirometry will be performed. (Note: subjects' have up to 3 attempts to FEV1 of ≥70% to continue on with this study.)

general physical examination along with nasal examination.

Nasal lavage performed.

Blood work obtained for sulforaphane level.

Nasal Allergen Challenge followed by monitoring explained below

Study completed.

Study Outcome Measures:
1. The Total Nasal Symptoms Score (TNSS), performed to determine adequate dosing for nasal challenge (visit 1) and during the nasal challenges (visit 2, 3). During the challenge the TNSS will be taken 30 minutes after nasal lavage, and then repeated at 5, 15, and 30 minutes then hourly to 4 hours post-challenge.
2. The peak nasal inspiratory flow (PNIF) performed at the up-dosing nasal challenge visit (visit 1) and during nasal challenges (visit 2 and 3). During the challenge, PNIF will be taken 30 minutes after the nasal lavage, then repeated at 5, 15 and 30 minutes after the challenge, then hourly to 4 hours post-challenge.
3. Biomarker measurements taken from nasal fluid collected by nasal sponge at visit 2 and visit 3 will include:
a) IL-4: 0 mintue, 5 minutes, 15 minutes, 30 minutes, then hourly to 4 hours post challenge
b) IL-5: 0 minute, 5 minutes, 15 minutes, 30 minutes, then hourly to 4 hours post challenge
c) IL-13: 0 minute, 5 minutes, 15 minutes, 30 minutes, then hourly to 4 hours post challenge
d) Eosinophil Cationic Protein (ECP): 0 minute, 5 minutes, 15 minutes, 30 minutes, then hourly to 4 hours post challenge
e) Tryptase: 0 minute, 5 minutes, 15 minutes, 30 minutes, then hourly to 4 hours post challenge

Study Regimen:
Study Medications and Placebo
Nasal Corticosteroids: Fluticasone propionate nasal spray prescribed as 2 sprays in each nostril daily will be the nasal corticosteroid of choice for the current study. Placebo will be normal saline based. Both fluticasone and placebo will be ordered through the VAGLAHS Research Pharmacy. For optimal control of nasal symptoms a treatment period of 1 week is required. Fluticasone will be given for 3 weeks in order to maximize effect.

Active Avmacol Enteric Coated Tablets:Water, HPMC, Sodium Alginate, Purified Stearic Acid, Ethylcellulose, Medium Chain Triglycerides, Oleic Acid, Triactin, Mineral Oil, Sodium Lauryl Sulfate, Titanium Dioxide

Avmacol, a blend of broccoli seed and sprout extracts contains the 2 essential ingredients needed for sulforaphane production: glucoraphanin and the myrosinase enzyme. Avmacol contains ~ 30 - 35 umoles sulforaphane per 2/tablet recommended serving.

Placebo Avmacol Coated Tablets:Water, HPMC, Triactin, Mineral Oil, Sodium Lauryl Sulfate, Titanium Dioxide

Concomitant Medications
Concomitant medication is defined as any medication, which is taken during the study. All prescription, herbal supplements, and over-the-counter drugs which are being taken by subjects upon entry to the study or which are taken during the study are regarded as concomitant treatments and will be documented. Basic medication use information will be confirmed at the time of screening, in order to determine if subject meets exclusion criteria regarding medications.

Rescue Medications
Subjects in the study are atopic. During the course of the study, subjects may develop nasal symptoms. They will be allowed to treat their symptoms with saline nasal spray. If their symptoms persist and they require additional medications, they will be discontinued from the study.

Prohibited Medications
The days below indicate last dose received prior to visit 1 and throughout the subjects' total participation time:

1. Topical Nasal Steroid use in the past 14 days,
2. Systemic steroid use in the past 14 days,
3. Oral antihistamine use in the past 5 days,
4. Intranasal antihistamine or cromolyn use in week prior to nasal challenge.
6. currently receiving allergy immunotherapy, or use anytime during the protocol.
7. short acting beta agonists in the past 6 hours.
8. nasal cromolyn in the past 7 days,
9. oral decongestants in the past 3 days,
10. antihistamine-decongestant tablets/liquids in the past 3 days,

Nasal Sprays:
Both the active fluticasone proprionate (50mcg) and the placebo will be obtained from the VA Research pharmacy.

Criteria for Premature Termination of the Study

Participant Withdrawal Criteria
In the best interest of the subject, participants may be prematurely terminated from the study for the following reasons:
1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up"
3. The participant takes any of the prohibited medications during the study.
4. The participant develops an adverse event that, in the opinion of the Protocol Chair (Principal Investigator) or the Safety Monitoring Board of the study, increases his/her risks if study participation continues.
5. The participant dies

Study Stopping Rules
The Data Safety Monitoring Board (DSMB) will be provided summaries of all adverse events on a regular basis. The DSMB will have the authority to terminate the trial at any time based on reported adverse events.

Study enrollment and ongoing study treatments will be suspended pending expedited review of all pertinent data by the VAGLAHS Institutional Review Board (IRB), the Data Safety Monitoring Board (DSMB) if a significant adverse event *as deemed by DSMB* occurs that is possibly related to the study procedures. The study may be terminated by the DSMB upon review of data with observations or findings that such action is necessary. (National Cancer Institute's Common Terminology Criteria for Adverse EventsVersion 4 (published May 28, 2009)).

Grade 1=Mild adverse event
Grade 2=Moderate adverse event.
Grade 3=Severe and undesirable adverse event
Grade 4=Life-threatening or disabling adverse event
Grade 5=Death

Screening Visit
At the screening visit, potential subjects are given a copy of the IRB-approved informed consent form for review and are invited to ask any questions or express concerns after reading the consent. The research study is clearly explained to the subject, including the rationale, risks, and benefits. The subject is asked to explain what he/she understands to be required in the study. The subject is informed that all participation is voluntary and that he/she is free to discuss the study with family or friends when considering enrollment. Subjects are told that only if the study protocol is fully understood should they sign the consent form. After the subject provides written consent, potential subjects' eligibility will be confirmed based on the determined inclusion/exclusion criteria. Once the subject has signed the informed consent form, a study investigator will also sign it, and the subject will receive a copy.

Procedures performed
Skin Testing: Epicutaneous skin testing is considered highly reliable, safe and widely used in diagnosing allergic rhinitis (37). After application of the specific allergen to the sterile tip of the applicator, the subject's skin will be gently lifted and the tip containing allergen will be applied. Along with application of the allergens mentioned, histamine and a saline control will be applied to subject's skin. Results will be interpreted 20 minutes following and will be considered positive if the wheal and erythema are at least 3mm larger than the saline control. For subjects who have an epicutaneous allergen skin or blood test on file completed by the VA Allergy Clinic within $\leq$ 1 year that confirms a positive to grass pollens the results will be used to proceed onto the up-dosing nasal challenge.

Nasal Challenge: Nasal Challenge is considered a safe and useful diagnostic tool in diagnosing causes of both allergic and nonallergic rhinitis *(38).* The nasal challenge chosen was based on repeated reliability using the TNSS and PNIF scores along with the biomarkers chosen for this study. (36)

Subjects will undergo an up-dosing nasal challenge performed at visit 1 in order to determine the threshold dose to be given at the subsequent nasal challenges (visit 2 and visit 3). During the up-dosing nasal challenge, the TNSS will be recorded immediately before each subsequent dose. Grass allergen extract (*c. cynodon, p. pretense or s. sorghum*,) will be used in the

challenge. For c. cynodon (Bermuda grass), dosing will start at 30 BU/ml and will continue with the following concentrations until a TNSS score reaches 7 or greater or PNIF is reduced by ≥50% from baseline reading: 300, 1000, 3000 and 10,000 BU/ml. *For p. pretense(Timothy grass), dosing will start at 300 and will continue with the following concentrations until a TNSS score reaches 7 or greater or baseline PNIF is reduced by ≥ 50% from baseline reading: 1,000,3,000,10,000 and 100,000 BU/ml. For s. sorghum (Johnson grass) dosing will start at 1:200,000 and will continue with the following concentrations until a TNSS score reaches 7 or greater or baseline PNIF is reduced by ≥ 50% from baseline reading: 1:20,000, 1:2000, 1:200, and 1:20 w/v.* Subjects will receive three sprays equivalent to 100 µl for each concentration into each nostril using an atomizer device, part of the standardized equipment at VAGLAHS for patients requiring nasal sprays during procedures applied every 10 minutes until maximal concentration or a TNSS score of 7 or PNIF reduction by ≥ 50% from baseline reading is achieved. Prior to subsequent challenges at visit 2 and 3, nasal lavage (SinusRinse) will be performed. A baseline PNIF and TNSS will be recorded 30 minutes following nasal lavage for visits 2 and 3. Nasal challenge with 100 µl of the final dose achieved at visit 1 will then be applied into each nostril. The PNIF and TNSS will be recorded following challenge at 5, 15, 30 minutes following challenge and then at 1 hour, continuing to record each hour for 4 hours. During these intervals, nasal sponges (Plastocell & Co.) will be applied into each nostril onto the nasal mucosa, beyond the nasal vestibule, and alongside the inferior turbinate with the use of a crock forceps (Phoenix surgical instruments, Harlow, UK) and a Thuddicum's nasal speculum (Phoenix surgical instruments, Harlow, UK). After two minutes of placement, the sponges will be removed and then added to 2 ml centrifuge tubes with indwelling .22µm cellulose acetate filters (Costar Spin-X, Corning, NY, USA) and placed immediately into ice before centrifuging within the hour. The liberated fluid will then be freezed at -80º until analysis occurs.

General physical examination: A physical examination to determine general health to include measurement of vitals and a cardiopulmonary examination will be performed in the VA GLA Allergy/Immunology Clinic by the attending physician. A *nasal examination* will be performed at every visit before any nasal challenge or lavage is performed. On Day 1, a urine pregnancy test will be performed for women of child-bearing potential.

The Immediate Total Nasal Symptom Score (TNSS): The TNSS has been used in prior interventional studies to evaluate different treatment modes for allergic rhinitis *(39, 40*, and 41). The score will consist of subjects rating 4 symptoms (nasal congestion/postnasal drainage, nasal pruritus, rhinorrhea, or sneezing) on a scale of 0 to 3, where 0=no symptoms present, 1=mild symptoms that do not interfere with activity, 2=moderate symptoms that are slightly bothersome symptoms with slight interference with activity and/or nighttime sleep, or 3=severe symptoms with interference with activity and nighttime sleep.

The Peak Nasal Inspiratory Flow (PNIF): the PNIF (Alliance Tech Medical) has been used in prior interventional studies involving allergic rhinitis (42, 43, and 44). Patients exhales fully and quickly after checking that an air tight seal covers the nose and seals the mouth.

Nasal Examination: Nasal examination will be performed at each visit prior to nasal challenge. Each nasal examination will include use of an otoscope with specula inserted into the nasal passage. Nasal passages and nasal turbinates will be inspected for color, swelling, exudate or bleeding.

Precautions:

Since allergen will be administered through nasal challenge, it is imperative that the study take place in a controlled environment that is prepared to treat hypersensitivity reactions. The VAGLAHS Allergy/Immunology clinic has the appropriate equipment and trained personnel to treat allergic reactions including anaphylaxis. A crash cart is available inside the clinic and epinephrine is readily available. The clinic is located within the VAGLAHS hospital where there are physicians trained in advanced cardiac life support (ACLS).

Visit Target Windows:
Subject visit times during the treatment phase of the protocol should be consistent, within -+10 days of targeted scheduled visit days.

Randomization:
This is a double blind randomized study. The subject randomization will be carried out based on a computer generated randomization list with a random blocked design of block sizes of 4 and 8. Only the study statistician and pharmacist will maintain the randomization list, the treatment administrators and outcome evaluators are blind to the randomization. The investigator performing and recording the results of the challenge will be blinded to the subjects assigned treatment arm.

PAYMENT OFFERED FOR PARTICIPATION

Twenty dollars ($20.00) will be paid to participants who complete up to the skin test procedure at visit 1, and do not meet study qualifications (skin test results determine that you not allergic to grass) to continue participation in the study.
Forty-five dollars ($45.00) will be paid to participants who meet study qualifications and complete visit 1.
Ninety dollars ($90.00) will be paid to participants who complete visit 2.
Ninety dollars ($90.00) will be paid to participants who complete visit 3.
Additionally, those participants who will comply with study protocol and complete all three study visits will receive the completion of study payment in amount of $75 at the end of the study.

Study Material and analysis:
Choosing cytokines mediators:
TH2 cytokines (IL4, IL15, IL13): These cytokines direct production of IgE antibodies which latter attach to mast cells.

Tryptase, Eosinophilic Cationic Protein (ECP): Tryptase is immediately released from mast cells upon activation. ECP marks presence of eosinophils which are involved in the delayed reaction of allergic rhinitis.

Both the above cytokines, tryptase and ecp have been found in nasal mucus and levels have been replicated in prior nasal challenge (36)

Analysis of biomarkers:
All the following analysis will be performed at the biomarker lab, UCLA Center for Human Nutrition. Measurements of IL-4, IL-5, and IL-13 will be performed using MSD Human TH1/TH2 7-Plex, Ultra-Sensitive Kit according to the manufacturer's instructions (MS6000 7 spot, Meso Scale Discovery, Maryland, USA). Plates will read using anMSD SECTOR® 6000 instrument. With this method, validation procedures demonstrated that the lower quantification limit for all cytokines was approximately 5 pg/ml, with an assay range of 5–5000 pg/ml in nasal secretions.

All measurements will be performed on undiluted nasal fluid samples in duplicate and reported as mean values, though cytokine levels will be standardized with ng/ml protein concentration. Tryptase and Eosinophilic Cationic Protein (ECP) will be measured through ImmunoCAP at the minimum level of detection of 2mg/ml. Each plate/Immunocap carousel is run with standards and curve controls.

Genomic DNA Isolation:
Peripheral blood will be drawn into a BD Vacutainer K2 EDTA tube (BD Biosciences; San Jose, CA). Genomic DNA will be extracted from blood using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich; St. Louis, MO) according to manufacturer's instructions. Briefly, 200 µl blood will be treated with Proteinase K followed by RNase for 2 min before lysing cells in a 55 deg C water for 10 min. DNA will be precipitated with 100% ethanol and bound to a spin column. After two washes, DNA will be eluted from column with TE buffer. TaqMan Polymerase Chain Reaction Genotyping of GSTP1 Ile105Val Genotyping will be performed using SNP Genotyping Assays for GSTP1 (rs1695) (Applied Biosystems; Foster City, CA) on a StepOnePlus Real-Time PCR Machine (Applied Biosystems). Real-time PCR will be performed with 25 ng DNA in a 20 µl reaction containing TaqMan Genotyping Master Mix and SNP genotyping assay. Reactions will be heated for 95 oC for 10 minutes following by 40 cycles of 92 oC for 15 sec with 60 oC for 1 min. Alleles will be determined using the allelic discrimination plot in the StepOne software genotyping program. Each sample will be performed in quadruplicate.

GSTM1 Copy Number-GSTM1 copy number will be determined using Pre-designed TaqMan Copy Number Assays (Applied Biosystems). GSTM1 will run in a duplex assay with RNase P as a reference gene. Reactions will be heated for 95 oC for 10 minutes following by 40 cycles of 92 oC for 15 sec with 60 oC for 1 min. Results will be analyzed using Copy Caller version 1.0 software (Applied Biosytems). Each sample is performed in quadruplicate.

Measurement of sulforaphane:
HPLC-MS/MS identification of SFN metabolites in plasma R, S-sulforaphane will be purchased from LKT Laboratories, Inc. (Minnesota, USA). All solvents and other chemical used will be HPLC grade and were purchased from Fisher Scientific (USA). Water will be ultrafiltered using a Milli-Q pure water system. To the 4° C plasma sample (200 µL) in a 1.5mL micro centrifuge tube (Fisher Scientific, USA), pre-cooled (4° C) trifluoroacetic acid (20 µL) (TFA) will be added to precipitate plasma proteins (30 seconds) in the sample followed by brief mixing and refrigerated centrifugation at 15900×g for 10 min. The resulting supernatant will be filtered (0.2 µm pore size) and 20 µL injected for analysis.

A LCQ Advance mass (Thermo Finnegan) and a Survey HPLC equipped with binary pump, degasser, cooled auto-sampler and a PDA detector (USA) were used for the LC–MS analysis. The HPLC column will be a Zorbax SB- will be 0.25 mL/min and gradient elution will be used: solution A (1% acetic acid solution): solution B (acetonitrile). The gradient started at 2% solution B increasing over 20 min to 30% B and 50% in 30 minutes, finally re-equilibrating to 2% B for 10 min. The mobile phase solutions were filtered through 0.22 µm pore size cellulose nitrate or nylon membrane filters using a vacuum filtration apparatus. The LC eluent flow of 0.25 mL/min will be sprayed into the mass spectrometer interface without splitting. Electrospray ionization in the positive mode (ESI+) will be applied. The analytes are identified by molecular weight and their MS/MS, respectively. The identification of the MS/MS of SFN-Cys, SFN-Cys-Gly; SFN-NAC; and SFN-GSH at m/z of 178; 179; 178 and 179, respectively were used to confirm consumption of the BSE supplement by subjects in the study.

Peak Nasal Inspiratory Flow (PNIF):
PNIF will be performed on each subjectthroughout nasal allergy challenge testing. Measurements will be performed using the In-Check Inspiratory Flow Measurement Device (Alliance Tech Medical), a portable inspiratory flow meter that can monitor nasal patency. Nasal peak inspiratory flow will be measured in L/min. The subject will place the device over the nose and mouth. The subject will be asked to take a deep, quick forced inspiration through the nose. The test will be repeated three times and the highest value will be recorded.

Statistical Considerations and Analytical Plan:
Summary statistics (mean, standard deviation, median, interquartile range and frequency distribution) will be generated for baseline demographic and clinical information to characterize the study population for subjects of 4 treatment arms, respectively. All outcomes of interest (TNSS, PNIF and biomarkers) will also be summarized by treatment arms and repeated measurement time points. Box plots and histograms will be generated to visualize the variable distributions, and scatter plot will be used to explore the relationship among variables. Primary analysis: outcomes of interest will be collected at different time points in visits 2 (baseline) and 3 (after 3 weeks of treatment). TNSS will be recorded before 30 minutes after nasal lavage, 5, 15, 30, mintues then hourly to 4 hours after challenge initiation. According to Bareille (figure 2), TNSS is stable after 3 hours following challenge, therefore we will average the TNSS at 3 and hour as the analysis outcome to stabilize the outcome measure and simplify the analysis.

We are interested in detecting the difference in the outcomes among subjects receiving a placebo tablet vs. active tablet , with and without nasal corticosteroid. The primary endpoint is the TNSS. ANCOVA (Analysis of covariance) model will be used to evaluate the difference in TNSS after treatment between placebo tablet vs active tablet , adjusting for their baseline values. Analysis will be carried out based on intention to treat principal with and without covariate adjustment. In addition to treatment assignment (4 groups), other fixed effects in the model may include baseline value, subject-level covariates (e.g., age, sex, baseline disease severity, medical history, etc.). Estimates of difference between placebo vs. active tablet will be calculated based on appropriate contrasts. Variable transformation (e.g., log transformation) will be used if the outcome distribution is skewed and normality assumption is imperative or a better model fit is suggested with transformed covariates. Secondary analysis will include the change in PNIF and biomarkers, and similar ANCOVA model will be used. Correlation analysis will also be carried out to examine the associations among outcomes.

Sample size consideration: Similar to the study design in Bareille 2012, we used standard deviation (SD) of TNSS of 2.08 to calculate the sample size needed. With a total of 152 subjects (36 subjects per arm) and the adjustment for multiple comparisons, we will have 80% power to detect a difference of 1.5 in TNSS between mango juice vs mango juice + BS groups at the 2.5% level with or without nasal corticosteroid. This calculation is based on 2 sample t-test with sample size of 36 per group, common standard deviation of 2.08 and alpha=0.025. The difference of 1.5 in TNSS is right in the range of difference between various treatments in Bareille 2012. Since TNSS outcome is taking average over 3, 4, 5 and 6 hours results, we expect the variability will be reduced and sample size calculation will be conservative. Similar calculation can be carried out for PNIF and Biomarkers. According to (Lipworth 2012), we estimate the standard deviation % change in PNIF is about 12.7, with the sample size of *36* per arm, we will have 80% power to detect a difference of *9.1* in % change in PNIF between placebo tablet vs active tablet at the 2.5% level with or without nasal corticosteroid. According to (Baroody 2013), we estimate the SD of Tryptase is about 5 in their FF/PL (fluticasone

furoate/placebo) group, and we will have 80% power to detect a difference of *3.6* at the 2.5% level.

We will use multiple imputation method to handle the missing data problem such that each missing value will be replaced with a set of plausible values that represent the uncertainty about the right value to impute. There multiply imputed data sets will be analyzed by the proposed analytic methods and the results from these analyses will be combined based on Rubin's formula (Rubin 1987, Multiple Imputation for Nonresponse in Surveys, New York: John Wiley & Sons, Inc.).

We will recruit 475 subjects that meet the inclusion/exclusion criteria for screening. We expect 237 subjects will test positive to grass and negative to perennial allergens, 190 subjects will have adequate positive nasal challenge and will be randomized. The expected dropout rate is 20%.

Scatterplots and Spearman correlation will be produced to assess correlations between increase/decrease levels of the above variables. We are looking for change biomarker variables that reflect inflammation, TNSS and PNIF. We will assume effective anti-inflammatory treatment will lead to a significant decrease in inflammatory markers (IL4, IL5, IL13 and ECP), a significant increase in Total Nasal Symptom Scores (TNSS) and Peak Nasal Inspiratory Flow (PNIF).