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Merck Investigator Studies Program Review Committee (MISP-RC) IIS Clinical Concept Form

All fields are required, an incomplete form will be returned to the submitter. If a field is not completed, please note the reason.

Proposed Study Title	
Study Title:	**Desensitization and Cross-Desensitization during Oral Grass or Ragweed Pollen Immunotherapy**
Request Date:	1/31/2015 or as soon thereafter as possible

Principal Investigator Contact Information	
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Contracting Information (if applicable)	
Name:	
Phone/Fax:	
E-mail:	

Study Information	
Indication	**Understanding desensitization and cross-desensitization during allergen SL immunotherapy.**
Phase:	Pre-clinical
Number of Subjects:	30

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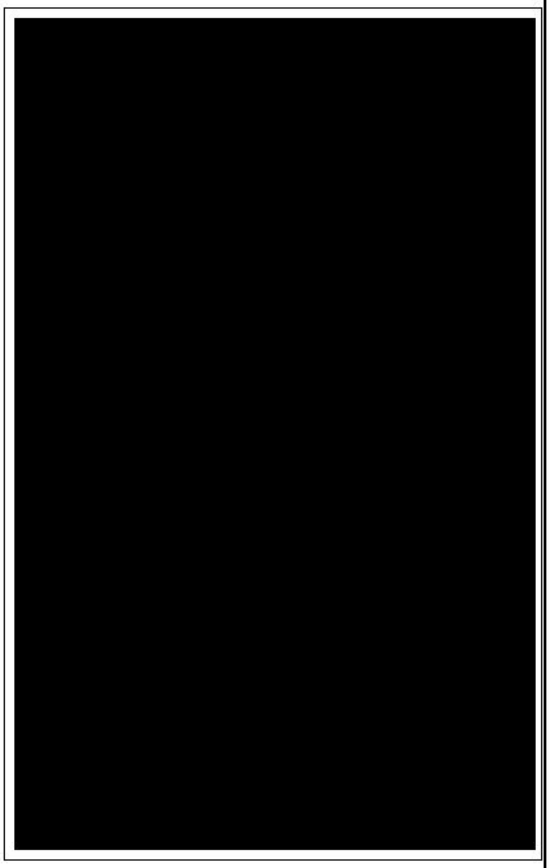
Background and Rationale

- Provide background on unanswered question(s) the study is attempting to answer (do not exceed one page)

During immediate hypersensitivity reactions (a.k.a. allergic reactions), cross-linking of IgE-bound FcεRI by antigens, including pollen proteins, food proteins, or self-proteins haptenized by medications, leads to rapid secretion of preformed and newly-synthesized mediators by mast cells and basophils. Depending on where mast cells are activated, these mediators can result in hives, angioedema, vomiting, diarrhea, bronchoconstriction, hypotension, and even death. Two principal immunologic phenomena have been exploited to prevent allergic reactions, from rhinitis to systemic anaphylaxis, in sensitive individuals: tolerance, a state of allergen-specific clinical unresponsiveness produced after years of treatment that lasts well beyond the duration of treatment; and desensitization, clinical unresponsiveness produced after hours of treatment that wanes soon after therapy is discontinued. Allergen-specific immunotherapy has been used to reduce the risk, severity, and duration of symptoms after exposure to inhaled allergens and insect stings by inducing long-term tolerance.[1,2] Procedures to induce clinical desensitization have been employed for nearly 70 years for medications and for more than a century for foods.[1-4]

Despite these empirical procedures being at the core of the clinical practice of Allergy & Immunology for many years, the mechanisms by which clinical unresponsiveness occur remain largely unknown. Experimental desensitization *in vitro* was demonstrated as early as 1995 to occur in guinea pig peritoneal mast cells[5] and in primary human mast cells by 1998.[6,7] In the latter study, first activating basophils or mast cells in calcium free buffer was necessary to achieve good desensitization, a practice not required for human desensitization. Also, desensitization appeared to result from the loss of antigen-specific IgE on the cell surface, because the ability of the mast cells to respond to antigen could be restored after incubating the desensitized cells with fresh antigen-specific IgE. Using a sequential-dose *in vitro* desensitization protocol, it was recently proposed that desensitization impairs the internalization of IgE/FcεRI complexes in cultured mouse bone marrow-derived mast cells.[1,2,8] More recently, our group has utilized two different approaches to study the mechanisms of antigen-mediated desensitization. The first is an *in vitro* sequential desensitization model in which cultured primary human mast cells are initially sensitized with antigen-specific IgE and then desensitized by the addition of increasing concentrations of allergen, from 1 pg/ml to 10 ng/ml added every 15 minutes.[1,2,9] The second is an *in vivo*/*ex vivo* model in which human volunteers, penicillin allergic by history and sensitive by skin testing, undergo an oral desensitization protocol consisting of ingestion of increasing doses of penicillin VK from 100 to 640,000 units every15 minutes. Titration skin testing and blood collection for *ex vivo* analysis of basophil function was performed before and after desensitization.



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Sublingual administration of medications first became established with development of sublingual nitroglycerin tablets for angina. In addition to the administration of small synthetic drugs, the mucosal surface also has been explored for vaccination strategies and introduction of small therapeutic proteins, such as insulin. For proteins, the sublingual route has the advantages of avoiding denaturation by the acidic gastric environment, degradation in the small intestine by pancreatic proteases, and hepatic metabolism when absorbed into the mesenteric circulation. Further, the placement of the protein(s) in tablets ensures prolonged contact with the sublingual mucosa, thereby enhancing absorption. Thus, tablet immunotherapy permits the gradual introduction of allergens or allergen-derived peptides either into the immune pathway through uptake by mucosal dendritic cells that then migrate to secondary gut-associated lymphoid tissue or into the vascular system to be distributed systemically. The former pathway is likely to be responsible for enhancing the production of Treg cells that could cause long-term antigen-specific tolerance, while the latter is likely to desensitize mast cells and/or basophils.

Cross-desensitization has been described in several biological receptor symptoms, though reports of this phenomenon in the context of FcεRI activation are few. In one report, cross-desensitization occurred in vitro with mast cells sensitized with both IgG and IgE. However, it has not been studied rigorously in a clinical setting, and thus the incidence of cross-desensitization among patients undergoing clinical desensitization or sublingual immunotherapy is unknown. As little is known about cross-desensitization, it is difficult to predict how this might affect the clinical practice of allergy. It is conceivable that the effect could be exploited to produce clinical benefit—if cross-desensitization could be attained during sublingual immunotherapy to a relatively innocuous allergen, perhaps it could be utilized to minize the risks associated with other forms of allergen-specific immunotherapy, such as oral immunotherapy to foods.

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References

1. Durham SR, Emminger W, Kapp A et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010;125(1):131-138.
2. Golden DB. Insect sting allergy and venom immunotherapy. *Ann Allergy Asthma Immunol* 2006;96(2 Suppl 1):S16-S21.
3. Edwards HE. Oral desensitization in food allergy. *Can Med Assoc J* 1940;43(3):234-236.
4. Browne SG. Desensitization for dapsone dermatitis. *Br Med J* 1963;2(5358):664-666.
5. Buckner CK, Ro J, Brendel J et al. Studies of desensitization and cross-desensitization to immunologic and nonimmunologic stimuli that evoke contraction and histamine release in superfused guinea pig trachea. *J Allergy Clin Immunol* 1991;87(3):655-661.
6. MacGlashan D, Jr. Desensitization of IgE-mediated IL-4 release from human basophils. *J Leukoc Biol* 1998;63(1):59-67.
7. MacGlashan D, Jr., Lavens-Phillips S, Katsushi M. IgE-mediated desensitization in human basophils and mast cells. *Front Biosci* 1998;3:d746-d756.
8. Sancho-Serra MC, Simarro M, Castells M. Rapid IgE desensitization is antigen specific and impairs early and late mast cell responses targeting FcepsilonRI internalization. *Eur J Immunol* 2011;41(4):1004-1013.
9. Zhao W, Gomez G, Macey M, Kepley CL, Schwartz LB. In vitro desensitization of human skin mast cells. *J Clin Immunol* 2012;32(1):150-160.

Objectives

- List the objectives to correspond directly with the listed hypotheses:

To induce clinical tolerance, a failure to respond to an allergen to which one was previously responsive, is an important objective for physicians, one that plays a significant role in the primary prevention of allergic reactions in the clinical practice of Allergy & Immunology. The tolerance resulting after standard subcutaneous immunotherapy to aeroallergen and insect venom allergens is long lasting and allergen-specific, and may involve antigen-specific T regulatory cells. In contrast, tolerance resulting from drug desensitization protocols is short-lived, and postulated to target mast cells and basophils. Research into the cellular and biochemical processes by which desensitization occurs has revealed that mast cells desensitized to one antigen in vitro, under certain conditions, lose the ability to degranulate to unrelated antigens or to direct FcεRI cross-linking. Preliminary data suggests that this cross-desensitization can happen in patients undergoing incremental desensitization, as described above, depending in part on the percentage of IgE targeted to the allergen used for desensitization. This proposal therefore aims to explore desensitization and cross-desensitization in human volunteers undergoing standard oral immunotherapy to grass or ragweed pollen.

OBJECTIVE I: Desensitization of cutaneous mast cells and blood basophils occurs during grass or ragweed pollen sublingual immunotherapy.

OBJECTIVE II: Cross-desensitization to unrelated allergens also occurs during grass or ragweed sublingual immunotherapy, depending in part on the portion of IgE targeting the allergen used for immunotherapy.

Hypothesis

- List the clinical Hypotheses in order of priority:

Objective I. We expect to see desensitization of mast cells and basophils to the allergen utilized during SL immunotherapy, but not to G protein receptor-coupled ligands, such as codeine and C5a. Moreover, it will be of interest to explore whether the magnitude of allergen-specific IgE levels in plasma affects the occurrence,

magnitude, or timing of desensitization. For example, it is possible that allergen-specific IgE in the serum serves to block or delay the arrival of allergen at the surface of mast cells, but perhaps not at the surface of circulating basophils. On the other hand, formation of low levels of small IgE:allergen immune complexes in the circulation might facilitate desensitization. Furthermore, whether desensitization reduces the level of free allergen-specific IgE, and such reduced levels of allergen-specific IgE correlates with desensitization, will be of additional interest.

Objective II: Based on our in vitro studies, showing allergen cross-desensitization of mast cell in culture, we expect cross-desensitization of mast cells to occur during SL allergen immunotherapy in most subjects, perhaps depending on the relative amount of the IgE against the desensitizing allergen. A similar incidence of cross-desensitization in basophils is expected based on their similar, but not identical, biology compared to mast cells. Learning how to safely cross desensitize may have important clinical utility, e.g., giving clinicians the ability to desensitize with ragweed SL tablets, and then safely begin immunotherapy with a more dangerous allergen, e.g., peanut or insect venom, or to administer a needed drug, e.g., penicillin or ciprofloxacin, to which the patient had been previously sensitized.

Study Design/Clinical Plan

- Provide a concise overview stating the type of experimental design

Central to both objectives of this proposal is the use of an approved sublingual immunotherapy protocol for allergen-specific immunotherapy. While our group has previously used increasing doses of swallowed penicillin in penicillin-sensitive patients to produce a state of clinical desensitization, the relatively low incidence of true penicillin allergy may make enrolling additional patients problematic. However, pollen allergic individuals are prevalent, and thus desensitization to such allergens is very feasible. Indeed, allergen-specific immunotherapy is considered standard of care for the treatment of allergic rhinoconjunctivitis. However, standard subcutaneous immunotherapy, administered by a weekly build-up followed by monthly maintenance injections is probably not ideal for desensitization. On the other hand, daily sublingual immunotherapy, as approved for patients with grass and ragweed allergic rhinoconjunctivitis, because of a relatively constant exposure, is more likely to result in desensitization.

Inclusion criteria: Adult subjects (18 to 65 years old, males and non-pregnant females) with a history of seasonal allergic rhinitis will be recruited for screening, which will include standard prick skin tests to: Timothy, Johnson, and Bermuda grass pollens; Oak, Ash, Hickory, and Mountain Cedar pollens; Short Ragweed pollen; *Dermatophagoides pteronyssinus* and *farinae*; and Cat and Dog allergenic extracts. The grass and tree pollens were chosen to minimize genus cross-reactivity, as described in: https://ainotes.wikispaces.com/file/view/Allergen+Cross+Reactivity+Table.pdf. Those who are sensitive to either Timothy or Short Ragweed pollen, and at least one but preferably two other allergens will be invited to participate.

Exclusion criteria: Subjects will be excluded for any one of the following: dermatographism or severe dermatologic condition, such as advanced eczema or psoriasis, that will not allow an adequate uninvolved area for skin testing; negative skin tests to Short Ragweed and Timothy or to at least one of the other listed aeroallergens tested; pregnancy; H1 receptor antihistamines taken within 7 days of testing; systemic steroids; omalizumab taken at any time; receiving or received immunotherapy; desensitized to any drug within 6 months; current uncontrolled or severe asthma; eosinophilic esophagitis; significant pulmonary, cardiovascular, renal, hepatobiliary, or neurological diseases, or another disease process felt to put subject at increased risk for an adverse event; hypersensitivity to any of the inactive ingredients in the tablet (fish gelatin and mannitol); mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with ability to comply with study requirements; or inability or unwillingness to give written informed consent.

Protocol: Subjects will undergo SL immunotherapy with either Timothy or Short Ragweed tablets (provided by Merck), taking one tablet per day, or will take a placebo tablet (provided by Merck). Titration skin testing to Timothy or Short Ragweed, to one or preferably two additional allergens to which the subject is sensitive, and

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to codeine as a control for mast cell activation capability through a non-IgE-dependent pathway will be performed to determine the PC3 value (see below). Skin testing, including histamine and diluent controls, will be performed prior to and at one and four weeks after initiation of immunotherapy. At each time point, blood will be obtained to measure total and antigen-specific IgE levels, tryptase and cytokine levels, and basophil activation with the relevant allergens and C5a as a non-IgE-mediated control for basophil activation.

First visit: Patients will be considered for this study if they report multiple (≥2) aeroallergen sensitivities, suggested by clinical history or previous skin or serum testing. Allergic sensitivities will be verified on the first visit by skin prick testing in the Clinical Research Services (CRS) unit in the North Hospital at Virginia Commonwealth University. Confirmation of Short ragweed or Timothy grass sensitivity and at least one, but preferably two, other unrelated aeroallergen sensitivities among the above list will be required for participation. Though there is conflicting evidence as to the correlation between wheal diameter from skin testing and the level of allergen-specific IgE measured in the serum, the allergen(s) with the largest wheal size will be selected for both the desensitizing allergen as well as for the allergens to test cross-desensitization.

Thirty subjects who qualify will then have blood drawn and undergo titration skin testing (see below) to the desensitizing allergen, to two other aeroallergens, and to codeine, from which PC3 values will be calculated. Wheal areas to histamine and diluent also will be determined. SL tablet immunotherapy will then begin with allergen tablets (n=15), as approved by the FDA, or placebo tablets (n=15) in a double-blind randomized manner.

Second visit: Patients will return to the CRS unit two weeks after SL immunotherapy has begun. At that time, titration skin testing will be performed to the same allergens plus codeine as on the First visit, along with a positive histamine and negative diluent control. Blood will also be collected for use in ex vivo analyses (see below).

Third visit: Patients will return to the CRS unit two months after SL immunotherapy has begun. At that time, titration skin testing will be performed to the same allergens plus codeine as on the First and Second visits, along with a positive histamine and negative diluent control. Blood will again be collected for use in ex vivo analyses (see below).

Subject Payments for Participation:

Visit 1: If potential subject fails screening, then no payment will be made. The individual will learn about their sensitivity to major aeroallergens. Upon completion of Visit 1, a $50 payment will be made.

Visit 2: Upon completion of Visit 2, a $50 payment will be made.

Visit 3: Upon completion of Visit 3, a $200 payment will be made.

Treatment

- List the clinical dosage/dosage form, route, and dose regimen:

The Timothy grass pollen allergen tablet (2800 Bioequivalent Allergy Units/tablet) (GRASTEK, Merck),[1] and the Short Ragweed pollen allergen tablet (12 Amb a 1-Units/tablet) (RAGWITEK, Merck)[2] have shown clinical efficacy. The current proposal will use either the Timothy grass tablet or the Short Ragweed tablet, along with a placebo tablet, supplied by Merck. The allergen tablet used will depend on which allergen the subject is sensitive to, which one corresponds to the strongest titration skin test response, and which one corresponds to an out of season allergen at the time enrollment is planned.

Subjects will be randomized to receive either allergen tablets (n=15) or placebo tablets (n=15) in a double-blind manner. The tablet will be placed under the tongue, where it should remain until fully dissolved, after which the patient should avoid ingesting food or beverages for five minutes. This first dose will be administered in the CRS unit, where the subject will be observed for 30 min, being monitored for any sign of an allergic reaction. If there is no clinically significant reaction, the subject will continue taking one tablet per day at home. Mild itching inside the mouth is expected to occur in a substantial number of subjects. This reaction should

generally subside within an hour, should generally cease after about one week, and is not considered to be clinically significant. If a clinically significant reaction does occur, such as systemic anaphylaxis or an asthma exacerbation, the subject will be withdrawn from the study.

Oxygen, epinephrine (IM), diphenhydramine (PO and IV), ranitidine (PO and IV), albuterol for nebulization, and a nebulizer will be available, if needed, in the CRS unit. A code cart will be available in the CRS unit, and a code button is present on each CRS bed. If a code is called, the dedicated code team of the hospital will respond (typically within 2-3 minutes) and transport the subject to an intensive care unit, as appropriate. Prior to the code team response, a code will be initiated by the personnel in place; both the CRS nurses and the Allergy/Immunology fellows are ACLS-certified.

References

1. Maloney J, Bernstein DI, Nelson H et al. Efficacy and safety of grass sublingual immunotherapy tablet, MK-7243: a large randomized controlled trial. *Ann Allergy Asthma Immunol* 2014;112(2):146-153.
2. Creticos PS, Maloney J, Bernstein DI et al. Randomized controlled trial of a ragweed allergy immunotherapy tablet in North American and European adults. *J Allergy Clin Immunol* 2013;131(5):1342-1349.

Collateral Research

- Include biomarkers, PK, etc.

Prick end point titration skin tests will be performed in duplicate with five 10-fold dilutions to commercially prepared aeroallergen extracts of Timothy grass or Short Ragweek and up to two additional environmental allergens, along with three concentrations of codeine (10, 30, and 90 mg/ml). Prick skin testing also will be done with a single concentration of histamine (10 mg/ml) and saline and will be administered simultaneously via a standard skin testing lancet. Skin test results will be recorded after 15-20 minutes by outlining wheals with a pen and then transferring the outline to transparent tape placed over the skin test sites. The tape records will be stored in a lab notebook as shown in **Figure 4**. The area of each wheal will be measured using image analysis software, and the mean area will be calculated as the average of those two measurements. The net wheal area will then be calculated by subtracting the mean of the duplicate negative control sites from the mean of duplicate allergen, codeine, and histamine skin test sites, and all net area values will be converted to net diameters assuming a perfect circle for each net area. The provocation concentration causing a 3 mm net wheal diameter (PC3) will then be calculated from a plot of the net wheal diameter versus the log allergen concentration, similar to previous publications using this technique.[1-3]

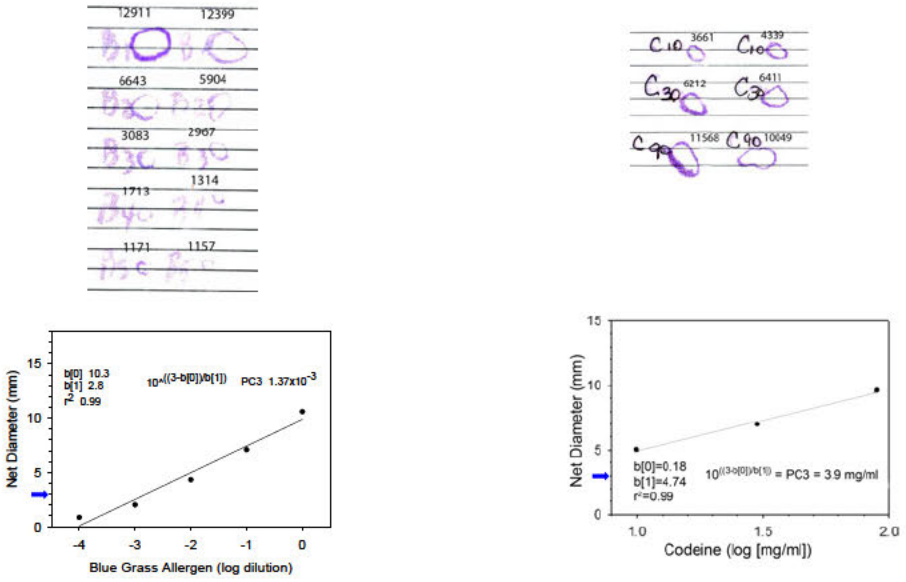

Figure 4. Titration skin testing and calculation of the PC3 value.

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Changes in PC3 after desensitization versus baseline and versus placebo will used to determine the degree of desensitization and of cross-desensitization. Codeine PC3 values are predicted to remain unchanged by allergen immunotherapy, as are the histamine positive and diluent negative controls.

Allergen-specific IgE to penicillin was not measurable in serum from any of the volunteers who underwent desensitization to penicillin in our previous study (data not shown), and thus we were unable to determine if clinically-apparent cross-desensitization was contingent on the amount of allergen-specific IgE or to the ratio of allergen-specific to total IgE, as it was for desensitization of human skin mast cells in vitro. To address this in the current study, aeroallergen-sensitive subjects will be recruited, in whom allergen-specific IgE as well as total IgE levels will likely be measureable on the UniCAP platform (ThermoFisher), permitting an analysis of any association between the absolute or relative amount of allergen-specific IgE to either desensitization or cross-desensitization.

Plasma and cells will be prepared from 100 ml of EDTA-anti-coagulated blood samples collected at each of the three visits. Plasma will then be assayed for total IgE, allergen-specific IgE, and tryptase levels using the Immunocap system (Thermo Fisher Scientific, Uppsala, Sweden). The ratio of allergen-specific to total IgE will be calculated for each time point.

To address the response of basophils to desensitization, basophils will be purified from the blood collected at each time point using protocols previously described. Some cells will be used to determine the baseline activation state of the basophils at each time point, as measured by CD63 or CD203c expression on the cell surface (flow cytometry). Other cells from each time point will be activated by incubation with an FcεRI cross-linking antibody, with purified allergens (ones used for titration skin tests) or with C5a, activation being measured by CD63 or CD203c expression. To determine if short-term changes in free allergen-specific plasma IgE correlate with the development of desensitization to allergen challenge, IgE levels at each time point will be compared to the above markers of mast cell and basophil activation (PC3 and CD63/203c expression, respectively).

References

1. Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;135(1):264-267.
2. Durham SR, Walker SM, Varga EM et al. Long-term clinical efficacy of grass-pollen immunotherapy [see comments]. *N Engl J Med* 1999;341(7):468-475.
3. Cockcroft DW, Davis BE, Boulet LP et al. The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. *Allergy* 2005;60(1):56-59.

Statistical Plans

- **Include justification for clinical sample size and primary hypothesis testing:**

Sample size consideration: Data is sparse on the effect of desensitization on threshold doses during percutaneous skin prick testing. Our previous experiments examined a change in PC3 values, but this was after a rapid, escalating-dose desensitization protocol, and no placebo group was included. Therefore, the proposed experiments have been designed to test the feasibility of measuring desensitization after single-dose sublingual immunotherapy using changes in PC3. A size of 15 subjects per group (treatment and control) was selected based on work recommending a sample size of at least 12 per group based on feasibility and precision about the mean and variance.[1] Moreover, this sample size should allow for adequate analysis of a secondary endpoint--the effect of the ratio of specific to total IgE on the degree of cross-desensitization--for which data is available. Based on published values of Timothy grass-specific to total IgE in immunotherapy patients[2] and the degree of cross-desensitization observed in our previous study, a sample size of 15 in the treatment arm should allow us to detect a 10-fold change in PC3 values per one-percent change in allergen-specific to total IgE ratio with 80% power and a 2-sided significance of 5%.

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Data analysis: The primary outcome for Objective I will be analyzed by comparing the change in PC3 values to Timothy grass or Short Ragweed between the placebo and active treatment groups using parametric (t test, repeated measures ANOVA) or non-parametric (Mann-Whitney, Friedman) tests, as appropriate for the data. Additionally, the proportions of each group showing clinically-significant desensitization to Timothy grass or Short Ragweed pollen, arbitrarily set at a 10-fold increase in PC3, will be compared using *Chi* square or Fisher's exact test. The primary outcome for Objective II will be analyzed by comparing the change in PC3 values to unrelated aeroallergen between both treatment groups, using the same methods as described above.

Secondary outcomes will be analyzed by comparing the percent IgE specific for Timothy grass or Short Ragweed, depending on the subject's initial sensitization, with the fold change in PC3 values after treatment for Timothy grass or Short Ragweed (Objective I) or unrelated aeroallergen (Objective II). Comparisons between continuous variables by be performed by calculating Pearson or Spearman coefficients or by regression modeling.

Subgroup analysis may be performed on any of the above measures if deemed necessary. For example, it may be determined that subjects treated with Timothy grass immunotherapy are more likely to display cross-desensitization than those treated with Short Ragweed immunotherapy.

All calculations will be performed using a statistical analyses software package such as SigmaPlot (Systat, San Jose, CA) or SPSS (IBM, Armonk, NY).

References

1. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceutical Statistics* 2005;4:287-291.
2. Niederberger V, Laffer S, Froschl R et al. IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE. *J Allergy Clin Immunol* 1998;101(2 Pt 1):258-264.

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Timelines and Study Plans

Number of Sites:	**One**
Site Names:	Virginia Commonwealth University Health System
Study Start Date:	Jan 31, 2015 or as soon thereafter as possible
Study End Date:	One year after start date
Number of Subjects:	30
First Patient In Date:	As soon after start date as possible

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Last Patient Out Date:	Two months before Study End Date
Enrollment Period in Months:	8 months
[redacted]	

Drug Supply Information	
Drug Supplies Required (Yes/No)?	**yes**
List Drug Supplies and Amount Required:	Drug Name: Timothy or Ragweed SL tablets Amount: 900 tablets in total, uncertain the distribution between the two
List Drug Supplies and Amount Required:	Drug Name: Placebo SL tablets Amount: 900 tablets
Placebo Required (Yes/No)?	**Yes**
Additional Sources of Drug Supply (Yes/No). If Yes, please specify	**No**