

Randomized, double-blind, placebo-controlled trial of standardized ragweed sublingual-liquid immunotherapy for allergic rhinoconjunctivitis

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Background: Sublingual immunotherapy with liquid extracts provides an appealing alternative to subcutaneous immunotherapy for the treatment of allergic rhinoconjunctivitis (ARC), but a lack of robust evidence has deterred its use in North America.

Objective: To determine the efficacy and tolerability of standardized glycerinated short ragweed sublingual allergen immunotherapy liquid (RW-SAIL) extract in subjects with ragweed-related ARC.

Methods: This phase 3, randomized, placebo-controlled trial was conducted in North America. Subjects (age range, 18-55 years) with or without asthma were selected based on ARC symptom severity and erythema skin prick reaction to short ragweed. Subjects self-administered the maximum tolerated dose of RW-SAIL (n = 218) or placebo (n = 211) daily beginning approximately 8 to 16 weeks before and through the end of the ragweed pollen season. The primary end point was subject-assessed total combined daily rhinoconjunctivitis symptom and medication scores (TCS).

Results: During the entire season, there was a 43% decrease in TCS in subjects treated with RW-SAIL compared with placebo. Similar decreases were observed in TCS between the 2 groups during peak season (42%) and in daily symptom scores during the entire (42%) and peak (41%) seasons. The occurrence of

adverse events was similar between the treatment groups; most were mild in severity. Treatment-related oromucosal local application site reactions occurred early and were transient and self-limited. No anaphylaxis occurred.

Conclusions: This is the first successful North American confirmatory phase 3 clinical trial to demonstrate the safety and efficacy of a sublingual standardized ragweed allergen immunotherapy liquid extract for the treatment of ARC. (*J Allergy Clin Immunol* 2013;■■■■:■■■-■■■.)

Key words: Allergen-specific IgE, allergen-specific IgG₄, allergy immunotherapy, combined score, ragweed, specific immunotherapy, sublingual immunotherapy

Subcutaneous immunotherapy has been the conventional mode of therapy in the United States and Canada for patients with seasonal allergic rhinoconjunctivitis (ARC) and milder asthma that is unresponsive to pharmacotherapy.¹ This effective form of treatment is burdened, however, by a prolonged injection schedule,² patient noncompliance due to frequent physician visits necessitated by the regimen,³ the discomfort associated with injections,⁴ and the recognized risk of anaphylaxis.¹ Sublingual immunotherapy (SLIT) represents an alternative mode of treatment that may afford a safe, convenient, and effective treatment modality for the management of allergic respiratory disease.^{2,5,6} Sublingual aqueous forms of immunotherapy have been espoused in Europe, and various studies have shown degrees of improvement with pollens and dust mite.^{4,7} However, SLIT is not approved by the US Food and Drug Administration (FDA), and it is used off-label with limited safety or dose-defining efficacy studies.⁸⁻¹³

Ragweed, the dominant seasonal aeroallergen for much of North America,¹⁴ causes significant morbidity, is associated with disease sequelae, and adversely affects the economic burden. The objective of this study was to demonstrate the efficacy and tolerability of SLIT with a standardized glycerinated short ragweed allergenic extract in adult subjects with ragweed-pollen-related ARC, with or without mild asthma. Here, we report the first successful confirmatory phase 3 clinical trial to demonstrate the tolerability and efficacy of a standardized ragweed sublingual allergen immunotherapy liquid extract (SAIL) (Greer SAIL, Greer Laboratories Inc, Lenoir, NC).

METHODS

Study design and oversight

This phase 3, randomized, double-blind, placebo-controlled, parallel-group clinical trial (NCT01353079) was conducted at 26 US and Canadian centers from April 2011 (before the natural ragweed pollinating season) until

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Abbreviations used

AE:	Adverse event
ANCOVA:	Analysis of covariance
ARC:	Allergic rhinoconjunctivitis
DSS:	Daily symptom score
FDA:	US Food and Drug Administration
ITT:	Intent to treat
LS:	Least squares
MTD:	Maximum tolerated dose
RW-SAIL:	Ragweed sublingual allergen immunotherapy liquid extract
SLIT:	Sublingual immunotherapy
TCS:	Total combined symptom score and medication score
TRAE:	Treatment-related adverse event

November 2011 (the end of the 2011 ragweed pollen season). Eligible subjects were randomized (1:1) by an interactive voice response system according to a computer-generated block-randomization scheme into ragweed SAIL (RW-SAIL) or to placebo groups. The study drug was administered sublingually, held under the tongue for up to 2 minutes, and then the residual was swallowed. Double blinding was ensured through use of a placebo identical to RW-SAIL in taste and appearance. Blinding was maintained until the database was locked. No fewer than 8 but no more than 16 weeks before the ragweed pollen season, all the subjects received a single subject-blinded placebo dose (Fig 1). The patients who reacted to that dose in the 15-20 minute follow-up were eliminated from the trial; those who could tolerate the initial dose received up to 2 incremental RW-SAIL doses (approximately 18 μ g Amb a 1 or approximately 50 μ g Amb a 1) or placebo at 15- to 20-minute intervals while being observed by the clinic staff. The dose was increased to the maximum tolerated dose (MTD) unless the subject experienced bothersome but tolerable symptoms. The MTD for each subject served as the subject's starting study dose. The MTD was self-administered once daily in the morning before food until the end of the ragweed pollen season. The subjects were provided with self-injectable epinephrine in case of a severe allergic reaction to treatment and as-needed antiallergy medications (loratadine 10 mg oral tablet once daily; olopatadine 0.1%, 1 drop twice daily) to use in a stepwise manner during the ragweed season for relief of moderate-to-severe ARC symptoms. Use of β -agonists was allowed for the subjects with asthma. The subjects were seen by the investigator every 30 days (± 7 days) during the treatment phase of the study. Treatment compliance was assessed by using daily electronic diary entries, and treatment vials were inspected during the monthly visits to confirm adherence to the dosing regimen. The subjects recorded each dose in their diary and were referred for further training if compliance dropped below 80%.

Ragweed pollen season was defined as starting on the first day in which ragweed pollen counts were >10 grains/ m^3 and ending on the first of 3 consecutive days in which ragweed pollen counts were <5 grains/ m^3 . The peak season was defined as the maximal 3 noncontiguous peak weeks of pollen counts over the course of the season for each study site, as determined by calculating a moving average of the available pollen counts for each week.

This study was conducted in accordance with the Declaration of Helsinki and the guidelines of the FDA and the International Conference on Harmonisation (Good Clinical Practices). The protocol was approved for each center by institutional review boards; written informed consent was obtained from all the subjects. The study sponsor and we were responsible for study design and/or data collection, data analysis, and manuscript preparation. We attest to the completeness and accuracy of the presented data.

Study population

Subjects, ages 18 to 55 years, with ragweed-related ARC, with or without mild intermittent asthma were included in the study. The subjects had a ≥ 2 -year history of moderate-to-severe ragweed-related ARC symptoms that required antiallergy medications. An orthogonal sum of erythema skin prick

test reaction ($\sum E$) ≥ 60 mm to short ragweed allergenic extract 15 to 20 minutes after application was required. Skin prick tests (Greer Pick; Greer Laboratories) were performed with allergenic extracts, saline solution, and histamine controls. Subjects with a history of anaphylaxis or a history of persistent or unstable asthma were excluded. Other exclusion criteria included ARC symptoms attributable to perennial allergens that overlapped the ragweed season, a history of symptomatic perennial ARC to an allergen to which the subject was regularly exposed, or inability to achieve dose 2 (approximately 18 μ g Amb a 1) or higher during preliminary dosing. Subjects who were polysensitized based on skin prick tests in the absence of symptoms were allowed in the trial.

Clinical and safety assessments

The primary efficacy measure was the net average daily rhinoconjunctivitis total combined symptom score and medication score (TCS) during the entire ragweed pollen season. The subjects recorded daily morning and evening symptoms and antiallergy medication use in an electronic diary. Ocular (itchiness, swelling and/or redness, and watery eyes and/or tears), nasal (sneezing, itching, runny nose, and stuffy nose), and aural (itching) symptoms were assessed and rated on an ordinal scale from 0 (no symptoms) through 3 (severe symptoms). Relief medication use was scored as 1 point for each daily use of oral or ocular antihistamine or albuterol treatment (excluding albuterol use before exercise). The TCS score was derived from the ocular (3 components) plus nasal (4 components) plus aural (1 component) (scored, 0-3; maximum symptom score, 24) plus the medication score (1 point for each medication use; maximum medication score, 3). The potential maximum TCS was 27.

Secondary efficacy variables were the TCS during the peak season, net average rhinoconjunctivitis daily symptom score (DSS) reported during the entire season, and the DSS during the 3 peak weeks of the season. Short ragweed specific IgG₄ and IgE antibody levels were exploratory end points evaluated from serum samples (ImmunoCap; Phadia AB, Uppsala, Sweden) collected at screening and the final study visit.

Safety was evaluated by adverse events (AE) self-reported by the subjects, concomitant medication use, vital signs, clinical laboratory evaluations, and changes in physical examinations. AEs were characterized for date and time of onset, duration, severity, and causal relationship with the study drug or other factors.

Statistical analysis

The intent-to-treat (ITT) population included all randomized subjects who received at least 1 dose of double-blinded study medication and who had at least 1 postdose efficacy assessment. The per-protocol population included subjects who met all inclusion and exclusion criteria, had no major protocol violations, and had at least 4 days of valid baseline data and at least 2 valid weeks of data during the entire pollen season. The safety population included all randomized subjects who received at least 1 dose of study medication. A sample size of 188 subjects in each group had 90% power to detect a difference of -1.44 in TCS means between the treatment groups by using a 2-group Satterthwaite *t* test with an SD of 2.9 and a 0.05 2-sided significance level. The sample size was increased by 10% to account for dropouts, which resulted in an estimated 209 subjects per treatment group. A clinically meaningful difference was defined as a 20% decrease in TCS in RW-SAIL versus placebo. Sensitivity testing for missing data for the primary end point included the use of the last-observation-carried-forward method by using the last data available before dropout on posttreatment assessments only, the use of the last-observation-carried-forward method on morning and evening symptom scores separately, examination of the rate of missing data in primary end-point scores between treatment groups, and the use of matched pairs.

Descriptive statistics were used for baseline, posttreatment, and change from baseline by treatment. Baseline symptom scores were determined during the week immediately before the ragweed season; at least 4 days of usable data were required. Efficacy end points were assessed in the ITT population. Analysis of covariance (ANCOVA) was performed to compare the active and

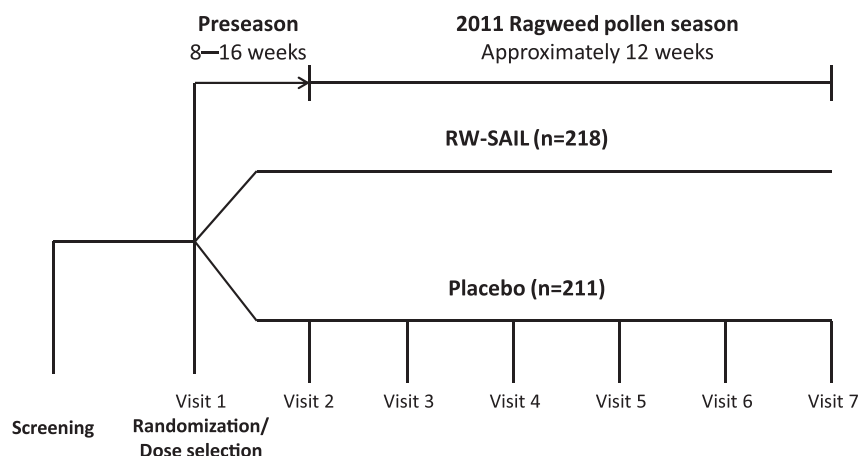


FIG 1. Study design. Determination of MTD occurred at visit 1.

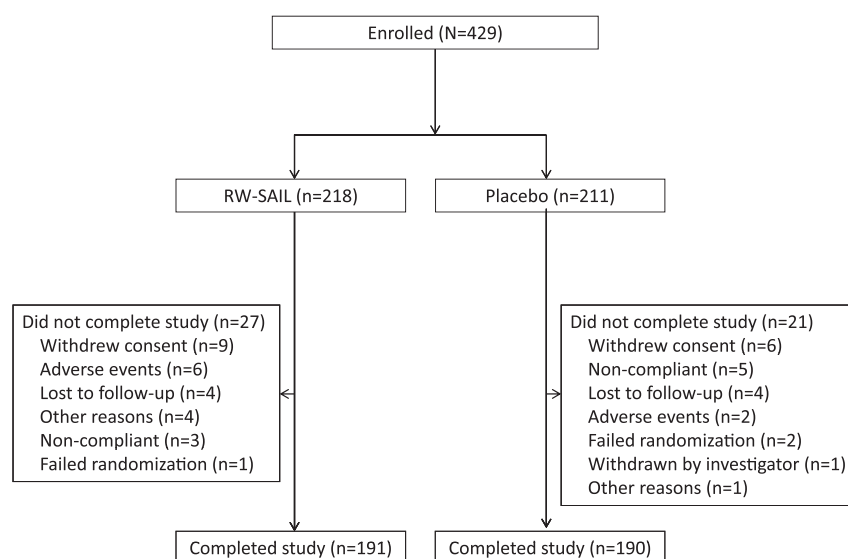


FIG 2. Enrollment and randomization of study subjects.

placebo groups. Change from baseline in TCS was used as the outcome variable for the ANCOVA with baseline TCS as a covariate. Treatment and study center were used as the independent variables. Least squares (LS) means for each treatment and treatment difference, as well as the 95% CI and *P* value, were calculated. Statistical analyses for secondary end points were done in a manner similar to TCS. Additional *post hoc* analyses were performed that served as sensitivity testing to confirm the robustness of the conclusions. ANCOVAs on the final scores (without baseline adjustment) and on baseline-adjusted scores were performed.

A *post hoc* analysis also was performed on the primary variable to test for a clinically meaningful difference between placebo and RW-SAIL based on a threshold applied to a lower bound of the 95% CI. Three cases were examined with $\delta = 10\%$, 15% , and 20% margins based on the mean TCS of placebo. Confidence bounds were based on a conservative 2-tailed approach.

RESULTS

Study participants

A total of 429 subjects were randomized (RW-SAIL, *n* = 218; placebo, *n* = 211); 381 subjects (88.8%) completed the study (RW-SAIL, *n* = 191; placebo, *n* = 190). The ITT and safety populations each totaled 429 subjects. Overall study compliance

was 89%. A total of 48 subjects (11.2%) discontinued in the study; the primary reason was withdrawal of consent (Fig 2). One subject in the RW-SAIL group and 2 subjects in the placebo group discontinued in the study because they failed randomization. A slight majority of subjects were women (56.9%), and the mean age was 38.3 years (Table I). Sensitivity to ragweed pollen was confirmed for all enrolled subjects (mean $\sum E = 94$ mm).

Ragweed pollen season

The mean duration of the 2011 ragweed season was 50 days (median, 49 days). The mean daily pollen count for the entire ragweed season was 44 pollen grains/m³ (peak ragweed season = 86 pollen grains/m³).

Primary efficacy end point

There was a significant improvement in the primary efficacy end point, TCS, during the entire ragweed pollen season, when RW-SAIL treatment was compared with placebo. The active-treatment group reported a 43% reduction in the primary end

TABLE I. Baseline characteristics of study subjects

Characteristic	Treatment		Total (N = 429)
	Placebo (n = 211)	RW-SAIL (n = 218)	
Age (y), mean (SD)	38.4 ± 10.1	38.2 ± 10.6	38.3 ± 10.3
Female, no. (%)	117 (55.5)	127 (58.3)	244 (56.9)
Race, no. (%)			
White	168 (79.6)	168 (77.1)	336 (78.3)
Black	28 (13.3)	34 (15.6)	62 (14.5)
Asian	11 (5.2)	9 (4.1)	20 (4.7)
Other	4 (1.9)	7 (3.2)	11 (2.6)
Weight (kg), mean (SD)	80.7 ± 21.5	81.7 ± 22.3	81.2 ± 21.9
Ragweed sensitivity			
Skin test reactivity (mm), mean $\sum$ E (SD)	96.0 ± 23.6	93.2 ± 20.4	94.6 ± 22.1
RW-specific IgE (kU/L), mean (SD)	14.6 ± 27.3	11.3 ± 18.9	13.0 ± 23.5
Asthmatics, no. (%)	19 (9.0)	17 (7.8)	36 (8.4)
Multisensitized, no. (%)	181 (85.6)	179 (82.1)	360 (83.9)

point relative to placebo recipients. The change from baseline in active treatment and placebo TCS was 0.82 versus 1.44, respectively (Table II); the median change was 0.45 versus 0.92 (difference in LS means, -0.83 [95% CI, -1.30 to -0.37]; $P < .001$). TCS scores paralleled the level of ragweed pollen over time (Fig 3). Analysis of the per-protocol population during the entire ragweed pollen season showed results similar to the ITT population.

Post hoc ANCOVAs on the final scores (without baseline adjustment) (see Table E1 in this article's Online Repository at www.jacionline.org) and ANOVA on baseline-adjusted scores produced similar results and supported the conclusions from the primary analysis. The adjusted lower bounds were higher than zero in all 3 cases, which indicated that, even after adjusting the 95% CIs to take into account a 10%, 15%, or 20% clinically meaningful threshold over placebo, the results from RW-SAIL remain statistically significantly lower compared with placebo (see Table E2 in this article's Online Repository at www.jacionline.org).

Secondary end points

The secondary efficacy end points significantly improved with RW-SAIL compared with placebo. Primary analysis of the peak season was based on the 3 noncontiguous "maximum" peak weeks. The subjects who received active treatment reported a 42% reduction in TCS during the peak season relative to placebo recipients. The change from baseline for subjects who received active treatment versus placebo at peak TCS was 1.17 versus 2.02, respectively (Table II); the median change was 0.67 versus 1.21 (difference in LS means, -0.86 [95% CI, -1.39 to -0.33]; $P = .002$). Similarly, subjects who received active treatment reported a 42% reduction in the DSS during the entire ragweed pollen season relative to placebo recipients. The change in the DSS from baseline for active treatment versus placebo was 0.79 versus 1.37 (Table II); the median change was 0.43 versus 0.91 (difference in LS means, -0.77 [95% CI, -1.21 to -0.33]; $P < .001$). The subjects who received RW-SAIL reported a 41% reduction in the DSS during the peak season relative to the placebo

recipients. The change in the peak DSS from baseline for active treatment versus placebo was 1.12 versus 1.90 (Table II); the median change was 0.65 versus 1.06 (difference in LS means, -0.79 [95% CI, -1.29 to -0.28]; $P = .002$).

Immunologic findings

There was a significantly greater increase in ragweed-specific IgG₄ antibody with RW-SAIL than with placebo (Fig 4, A). The change ($\pm$ SE) from baseline for active treatment versus placebo was 0.99 (± 0.13) versus -0.01 (± 0.01) mg/L, respectively (Fig 4, B); the median change was 0.21 versus -0.03 (difference in LS means, 0.94 [95% CI, 0.69 - 1.19]; $P < .001$). RW-SAIL also induced a significantly greater increase in ragweed-specific IgE antibody than in placebo (Fig 4, C). The change from baseline in active treatment versus placebo was 55.92 (± 10.21) versus 4.97 (± 1.18) kU/L (Fig 4, D); the median change was 11.47 versus 0.46 (difference in LS means, 41.41 [95% CI, 24.32 - 58.49]; $P < .001$).

Safety

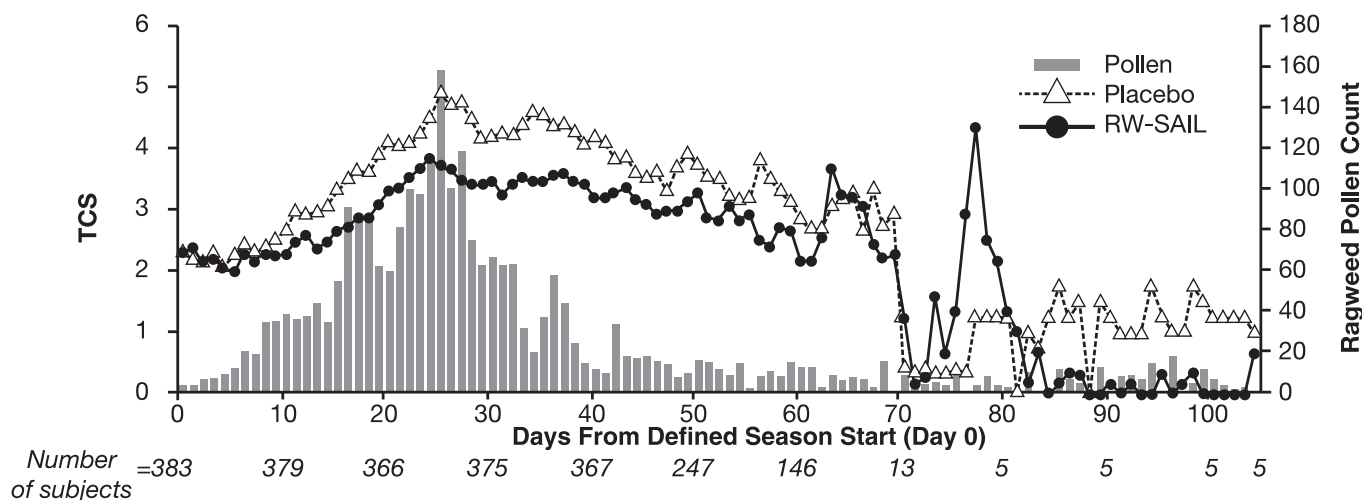
Ninety-four percent of subjects achieved the MTD (approximately $50 \mu\text{g}$ Amb a 1); 6% of the subjects either remained on, or stepped down, to the lower dose (approximately $18 \mu\text{g}$ Amb a 1). RW-SAIL was well tolerated, with no deaths, systemic allergic reactions, anaphylaxis, or life-threatening events. The percentage of subjects who reported AEs was similar throughout the entire study for the active and placebo groups (48% and 44%, respectively). Most AEs (95%) were mild or moderate in severity. The most common treatment emergent AEs were headache and upper respiratory and gastrointestinal symptoms (Table III). The percentage of subjects who reported treatment-related AEs (TRAE) was slightly higher throughout the entire study for the active-treatment group compared with the placebo group (12% vs 3%, respectively). The most frequently reported TRAEs were early occurring, transient, self-limited oromucosal reactions. Serious AEs occurred in 8 subjects (6 events in 3 subjects in the placebo group, 2 events in 2 subjects in the active-treatment group); none were considered treatment related by the study investigators. No clinically significant findings were recorded for laboratory parameters, except 1 subject with posttreatment emergent hematuria, judged not related to the study drug by the clinical investigator. A 25-year-old woman in the RW-SAIL group developed subcutaneous angioedema of the hand after contact with a cleansing product and was administered epinephrine in the emergency department. The AE was judged as not serious, was graded as mild, resolved in 2 days, and was considered probably not related to the study drug by the investigator.

DISCUSSION

To our knowledge, this is the first confirmatory phase 3 clinical trial to demonstrate the clinical efficacy of SLIT with a liquid allergenic extract (SLIT-liquid) in North American subjects with ARC. RW-SAIL significantly improved the primary efficacy end point and each of the secondary end points of the study throughout the entire season as well as the peak ragweed season. As further confirmation that meaningful clinical significance can be assigned to the statistical findings (mean TCS improvement with RW-SAIL over placebo), we also performed a lower-bound CI

TABLE II. Summary of RW-SAIL and placebo in average daily total combined symptom and medication scores and daily symptom scores during the ragweed pollen season

	Total combined score			Daily symptom score		
	Placebo	RW-SAIL	<i>P</i> value*	Placebo	RW-SAIL	<i>P</i> value*
Entire pollen season, no. patients	211	218		193	196	
Score, mean (SD)	1.44 ± 2.40	0.82 ± 1.64	<.001	1.37 ± 2.29	0.79 ± 1.56	<.001
Peak pollen season, no. patients	196	196		193	196	
Score, mean (SD)	2.02 ± 3.20	1.17 ± 2.10	.002	1.90 ± 3.01	1.12 ± 1.99	.002

P* values designate difference in LS means.FIG 3.** Mean TCS during the entire ragweed pollen season. Days 72-104 of the graph display data from only 3 centers (with data from only 4-13 subjects; only 1 center continued to supply data after day 77). The uptick in TCS seen in the RW-SAIL arm of the study on days 76-79 comprises data from only 3-6 subjects who recorded symptoms.

analysis. This lower bound analysis was performed by using 10%, 15%, and 20% as clinically relevant margins, and the upper bounds for all were <0 , which demonstrated statistical and, more importantly, clinical significance at the specified deltas (Table E2). In addition, the results from the current trial are comparable with those reported with other liquid or tablet SLIT studies^{7,11,15-19} that assessed TCS and immunologic end points. In the United States, SLIT is not approved by the FDA. However, increasingly, aqueous allergen extracts approved for subcutaneous administration are being administered off-label in the United States. This crystallizes the relevance of this clinical study because only limited data from safety and dose-defining efficacy studies exists to support this use.

Four studies have been reported in the literature with aqueous ragweed, none of which provide conclusive evidence of meaningful clinical effectiveness.^{11,13,20,21} The study by Andre et al²⁰ could be considered the first demonstration that ragweed extract can be administered as an aqueous material, although most subjects were converted to a tablet. Statistical significance in that study, which was not achieved for the primary end points (individual symptoms), was only achieved for secondary end points (physician global assessments and patient global assessments) that are not currently accepted as rigorous clinical outcomes for assessment by regulatory agencies.²⁰ The study by Bowen et al²¹ demonstrated a trend toward improvement in the primary end points of the study, sneezing ($P = .09$) and nasal pruritus ($P = .06$). Significance ($P = .04$) was only achieved for these

end points after exclusion of data from 1 study center because of a low pollen peak.²¹ The study by Esch et al¹³ was a phase 1 safety study performed on small numbers of subjects ($n = 9$ to 32) to establish the MTD in subjects allergic to ragweed, grass, dust mite, and/or cat, and did not provide efficacy data. The study by Skoner et al¹¹ was a dose-ranging study (48 vs 4.8 μg Amb a 1 vs placebo) that helped define the optimal dose for our clinical trial. This article did not find a statistically significant improvement in the ITT population for the primary end point, the rhinoconjunctivitis score (15% difference for active treatment vs placebo [$P > .1$ by ANOVA]). Only when subgroup analysis was performed on the group in the high-dose arm of the study could an effect be demonstrated ($P = .02$ by ANCOVA) for combined medication-symptom scores when compared with placebo.¹¹ Clearly, none of these studies provide pivotal evidence of efficacy with ragweed immunotherapy when it is administered as an aqueous sublingual extract.

In the United States, studies of SLIT-liquid with other allergens have been limited in size and aimed to establish safety (phase 1), insight into mechanism, or exploration of dose.^{8-10,12} As importantly, a review of the recent literature^{7,17,22,23} shows that the largest reported clinical studies of SLIT-liquid are less than half the size of our trial. Therefore, our study findings represent a major statement, not only for ragweed but also for SLIT as an entity.

We recognize that SLIT also can use dissolvable tablet formulations (SLIT-tablet).^{16,18-20,24-26} However, we would point out that the distinction between SLIT-liquid and SLIT-tablet is

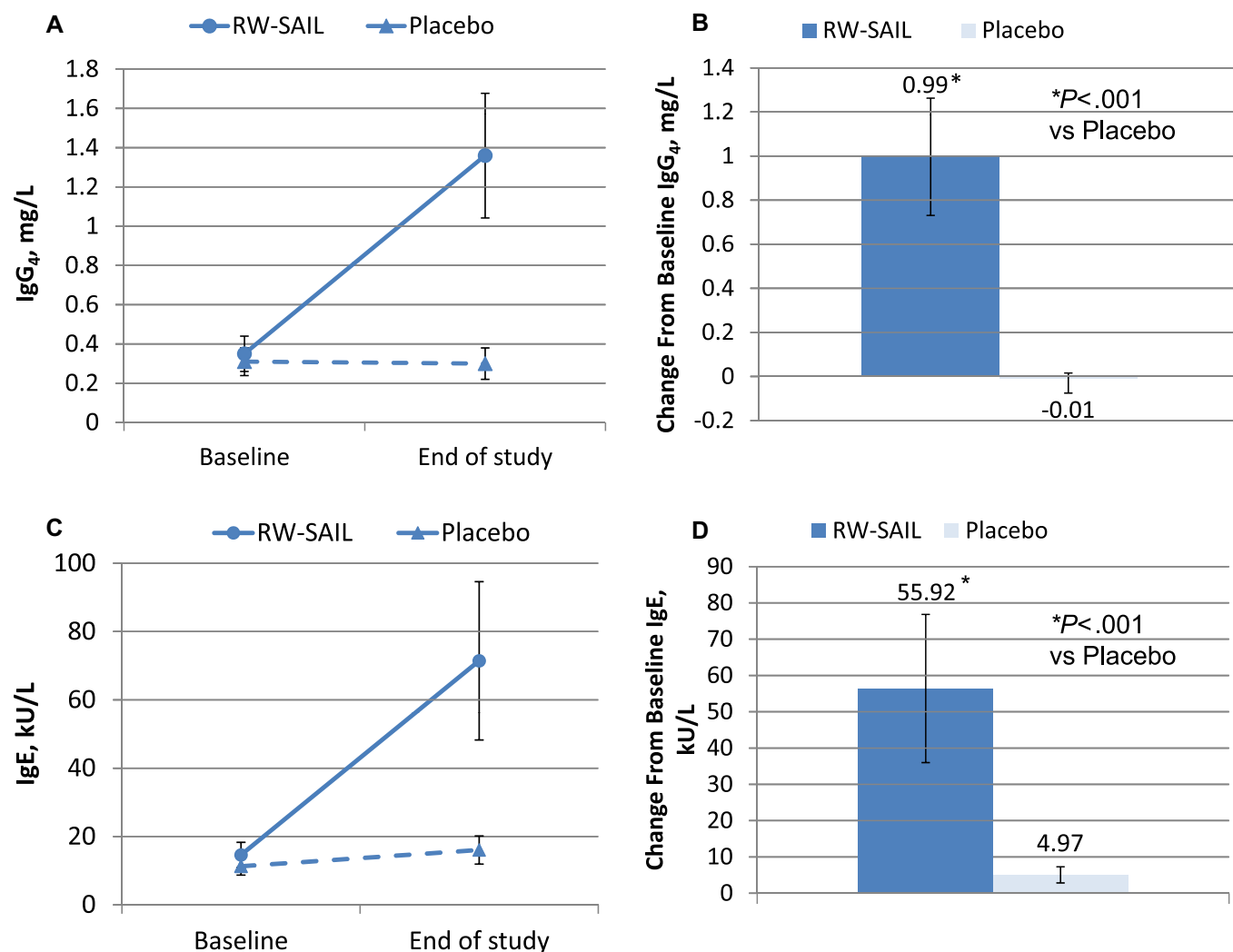


FIG 4. IgG₄ at baseline and end of study (A), IgG₄ change from baseline (B), IgE at baseline and end of study (C), and IgE change from baseline (D). Error bars indicate 95% CIs. *P* values designate difference in LS means.

important for several reasons. The 2 treatment formulations have different modes of delivery (aqueous extract vs dissolvable tablet) and may possess different absorption characteristics that could lead to different safety and efficacy profiles. In addition, the liquid formulation is currently FDA approved for subcutaneous immunotherapy and the clinical trials are aimed at providing sufficient evidence of safety and efficacy based on current FDA regulatory agency requirements for phase 3 pivotal trials to extend the use of the material to SLIT.

The complexities and difficulties inherent in conducting clinical trials that evaluate the efficacy of single allergen treatments in polysensitized subjects are evident from the findings in several recent reports.^{9,11,21} In this context, subject screening, inclusion-exclusion criteria, and confounding allergen exposure in the relevant allergen season are likely critical factors that reduce statistical power to detect meaningful differences in clinical trials.²⁷ In this trial, we attempted to address each of these potential study design flaws. Our study design used stringent screening to identify subjects who were highly allergic to ragweed based on clinical history and medication use, and

avoided geographic areas where confounding allergens were historically present or overlapped with the ragweed season. In particular, we used a higher threshold for study inclusion based on skin prick test $\sum E$ and not wheal, which has a flattening dose-response curve. This latter criterion represents a major difference in patient selection criteria compared with other trial designs. The determination of the $\sum E$ cutoff point and subject stratification was based on the methodology used in previous clinical trials initiated by the lead author (Peter Creticos) and retrospective analysis of subject-group skin test stratification from previous trials of RW-SAIL performed by the sponsor.^{11,13} Interestingly, Casale et al²⁸ recommended patient selection based on a combination of skin test sensitivity and ragweed-specific IgE, and recent clinical studies of SLIT-tablet indicate that IgE is a critical determinant in identifying symptomatic patients.^{15,16,18} In contrast, our findings clearly highlight the importance of *in vivo* methodology for ensuring robust clinical measurements.

The exposure to confounding allergens in polysensitized subjects in the context of allergen immunotherapy trials designed to prove efficacy toward a single allergen can have a significant

TABLE III. Most commonly reported TEAEs in study subjects, events reported by $\geq 2\%$ of subjects in either group

TEAE	Placebo, no. (%) (n = 211)	RW-SAIL, no. (%) (n = 218)
Headache	20 (9)	17 (8)
URI	22 (10)	11 (5)
GI disorder	13 (6)	18 (8)
Abdominal discomfort and/or pain	4 (2)	3 (1)
Constipation	0	4 (2)
Diarrhea	7 (3)	5 (2)
Dyspepsia	2 (1)	4 (2)
Oromucosal	5 (2)	22 (10)
Edema and/or swelling	0	12 (6)
Pain	6 (3)	5 (2)
Pruritus	0	5 (2)
Nasopharyngitis	9 (4)	12 (6)
Skin	8 (4)	10 (5)
Sinus and/or nasal congestion	10 (5)	4 (2)
Muscle strain	7 (3)	6 (3)
Back pain	7 (3)	6 (3)
Ear disorder	1 (0.5)	4 (2)

GI, Gastrointestinal; URI, upper respiratory infection.

impact on the outcome. The selection of subjects that are predominantly sensitized to the relevant allergen and the avoidance of geographic areas where overlapping and confounding allergens have been historically present can help but is not always feasible. In our previous ragweed SLIT trial,¹¹ we attempted to isolate the impact of ARC symptoms caused by confounding allergens on efficacy measures reported during the ragweed pollen season. The contribution of the symptoms induced by non-ragweed pollen allergens was estimated by recording symptoms during the week immediately before the ragweed season (baseline symptom score). We then used the baseline-adjusted symptom score to estimate the magnitude of the symptoms attributed to ragweed pollen exposure during the season. The use of this end-point measure greatly improved the statistical power by reducing the variability of symptom scores caused by subjects with sensitivities to the confounding allergens. This is the preferred statistical method of analysis when estimating a treatment effect, as opposed to ignoring baseline behavior altogether, and is especially valuable when baseline values are not negligible. This approach also may lead to an apparent enhanced treatment effect. For example, use of the baseline-adjusted scores predicted a 43% improvement in symptom-medication scores with respect to placebo, whereas use of the unadjusted scores predicted less improvement (calculated from Table E1). Given that the majority of the subjects included in this trial were polysensitized and that they were treated only for their ragweed allergy, the apparent difference of outcomes can be resolved. Further studies by using multiallergen immunotherapy in polysensitized subjects are needed to fully evaluate these effects. RW-SAIL increased IgG₄ and IgE significantly more than placebo, which is consistent with immunologic data observed in previously published clinical trials of subcutaneous immunotherapy^{29,30} and SLIT.^{16,18,20,31} Increases in IgG₄ were associated with decreases in ARC symptoms and medication use, which indicated that IgG₄ may be a protective biomarker.

During the 36-week treatment period, RW-SAIL was generally well tolerated. There were no deaths or anaphylaxis during this study, and few subjects (n = 8) withdrew because of AEs, all of

which were not unexpected (eg, diarrhea, local oromucosal AEs). The AEs reported in the study were mainly mild-to-moderate application-site reactions that are consistent with those reported in other SLIT trials of pollen allergens.³²⁻³⁴ No serious local or systemic allergic reactions were observed, which corroborated recommendations that SLIT is safe for at-home administration.³⁵ In conclusion, this North American clinical trial provides the first confirmatory evidence that once-daily SLIT-liquid administered to individuals with ragweed allergy is well tolerated and can result in highly significant clinical improvements in seasonal symptoms and rescue medication use. SLIT may provide a promising alternative therapeutic option for the treatment of patients with ARC.

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Clinical implications: The results of this study provide evidence that aqueous SLIT is well tolerated and effective for the treatment of ragweed allergy in a large North American population.

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Supplemental Material

Methods

An exploratory post hoc lower bound analysis was also performed at the request of the FDA on the primary variable. The purpose of this analysis was to examine whether the difference in TCS for RW-SAIL versus placebo was not only statistically and clinically significant as per the a priori Statistical Analysis Plan, but also to examine the clinical relevance of that difference when using different thresholds to define the margin. To test for a clinically meaningful difference between RW-SAIL and placebo based on a threshold applied to a lower bound of the 95% confidence interval (ie, upper bound for the negative difference), 3 cases were examined: 10%, 15%, and 20% margins based on the mean of placebo.

Results

For the lower bound analyses, the adjusted upper bound was -0.201 when the 10% pre-defined difference was used. When using the 15% difference, the adjusted upper bound was -0.116 , and when using the 20% difference, the adjusted upper bound was -0.032 (**Table E2**). All confidence limits listed above were based on a conservative two-tailed test. The adjusted upper bounds were less than zero in all 3 cases, indicating that even after adjusting the 95% CI to take into account a 10%, 15%, or 20% clinically meaningful threshold over placebo, the results for RW-SAIL remain statistically significantly lower than the results for placebo.

Discussion

The lower bound confidence interval analysis provides a strong indicator for determining the clinical relevance of a drug's therapeutic efficacy when compared with a population treated with placebo. The margin of difference observed in this clinical trial demonstrates a clear differentiation of results with treatment with RW-SAIL versus placebo.

Table E1. Summary of Change From Baseline in RW-SAIL and Placebo in Average Daily Total Combined Symptom-Medication Scores During the Ragweed Pollen Season

Post hoc Baseline Unadjusted ANCOVA Analysis

	Total Combined Score (TCS)*			Daily Symptom Score (DSS)*		
	Placebo	RW-SAIL	<i>P</i> Value [†]	Placebo	RW-SAIL	<i>P</i> Value [†]
Baseline Score	2.11±2.74	2.17±2.73		2.06±2.68	2.09±2.61	
Entire Pollen Season	n=211	n=218		n=193	n=196	
Score	3.55±3.40	2.99±3.00	<0.01	3.42±3.30	2.88±2.90	<0.01
Peak Pollen Season	n=196	n=196		n=193	n=196	
Score	4.13±3.81	3.31±3.24	<0.01	3.96±3.65	3.20±3.14	<0.01

LS=least squares; RW-SAIL=ragweed sublingual allergen immunotherapy liquid extract.

*Mean ± SD.

[†]*P* values designate difference in LS means.

9 **Table E2. Lower Bound Analysis**

Lower Bound CI Difference*	Adjusted Upper Bound
@10%	−0.201
@15%	−0.116
@20%	−0.032

10 CI=confidence interval.

11 *Confidence limits based on a conservative two-tailed approach.

12

**A RANDOMIZED, DOUBLE-BLIND, PLACEBO-
CONTROLLED PARALLEL GROUP EFFICACY AND
SAFETY TRIAL OF SUBLINGUAL-ORAL
IMMUNOTHERAPY (SLIT) IN ADULTS WITH ALLERGIC
RHINOCONJUNCTIVITIS CAUSED BY RAGWEED
POLLEN.**

Development Phase: Phase III

Investigational Product: Standardized Glycerinated Short Ragweed Pollen Allergenic Extract

Indication: Sublingual-Oral Immunotherapy

Sponsor: Greer® Laboratories, Inc.
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Allergy & Asthma Specialist of Greater Washington Centers
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Warrenton, Virginia 20186

Protocol Number: SLIT 10-01

Protocol Version: v. 8.0: August 12, 2011

Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) and regulatory requirements as applicable.

PROTOCOL FLOWCHART

Screening

N ≥418: Obtain informed consent and medical history; Screen subjects by criteria; Perform physical exam, pulmonary function test, urine pregnancy test (when applicable), allergen skin prick tests, and collect blood for assays.



Randomization & Stratification

209 Subjects
Treatment
Group

209 Subjects
Placebo
Group



Preliminary Dosing: Visit 1

Perform dose escalation regimen to determine MTD
Pre-seasonal treatment 8-16 weeks

Treatment Course: Visits 2 - 6

Administer Study Product/Intervention

Self-administer SLIT dose once daily. Record daily symptoms and allergy relief medication use in an e-diary. Clinic visits occur every 4 weeks for clinical and AE assessments. Dose adjustments are made as deemed necessary.

Post-Treatment Visit 7

Post-Treatment Visit

Perform physical exam and urine pregnancy test (when applicable); Collect blood for assays; Collect study drug, e-diary, Subject Notebook; Discharge Subjects or follow Subjects until untoward symptoms resolve.



**Assessment of Final Study
Outcome Measures**

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

The primary aim of this clinical trial is to demonstrate the efficacy and safety of Greer® Standardized Glycerinated Short Ragweed Pollen Allergenic Extract administered by the sublingual-oral route and to support changes in labeling for the currently licensed Greer® Standardized Short Ragweed Pollen Allergenic Extract.

Specific allergen immunotherapy as currently practiced in the USA and described in product labeling comprises the subcutaneous injection of incrementally increasing doses to a targeted maintenance dose (“build-up”) followed by maintenance injections of allergenic extract/vaccine. Up to 30-40 injections may be required during the build-up phase over a 3-6 month period. When adequate maintenance doses are reached, this form of immunotherapy (SCIT) has been shown to be highly effective and safe [1-2]. Standard practices recommend that the injections be given under the supervision of trained physicians and that the patient remain in the physician’s office at least 20 to 30 minutes after an injection. The administration of immunotherapy injections are not recommended at home because of the risk of inadequate recognition and treatment of systemic reactions [3]. The inconvenience and expense of traveling for allergy injections and the discomfort of the repeated injections is a disincentive to this form of treatment particularly in pediatric patients. For example, dropout rates exceeding 50% over a multi-year course of injection treatment have been reported [4].

Alternative routes for immunotherapy have been explored, especially in Europe in an attempt to improve patient compliance and to minimize the risk of serious adverse reactions [5]. For example, sublingual-oral immunotherapy (SLIT), which is the administration of the allergenic extract/vaccine under the tongue for 1-2 minutes followed by swallowing, has been proven to be efficacious and safe in several double-blind, placebo-controlled studies. A recent Cochrane Review concluded, “SLIT is a safe treatment, which significantly reduces symptoms and medication requirements in allergic rhinitis.” [6] Efficacy studies support the use of SLIT for the treatment of rhinitis or rhinitis and asthma [7-15].

Much of the United States medical community's hesitation to embrace sublingual immunotherapy as a viable treatment option for allergy patients has stemmed from limited information using U.S. licensed allergenic extracts for this treatment route. Additionally, the cost-effectiveness of one form of therapy over the other has not been clearly evaluated [16-17] and the third-party payers have not accepted SLIT for insurance coverage [18].

The safety profile reported in studies from Europe [19-24] need to be confirmed using US licensed allergenic extracts. For example, current safety data suggests that SLIT can be administered at home, making this therapy accessible to more patients. The most frequently reported side-effect was oral itching followed by gastrointestinal complaints. Oral itching was always reported as mild and self-resolving. Gastrointestinal complaints were associated with doses greater than 100 times that given during SCIT. Rhinitis, conjunctivitis, tiredness, and urticaria have been reported less frequently and their incidence (0.01 – 0.05%, or 1 to 5 per 10,000 doses) did not differ from the placebo group. The rate of systemic adverse events with SCIT ranges between 0.1 to 1% (10 to 100 per 10,000 doses) of which 5-10% may be severe [25-27]. The safety profile of SLIT is expected to be superior to the subcutaneous route even when significantly higher doses of allergenic extract are administered.

A Phase I/II safety and dosing study with Greer® Standardized Short Ragweed Pollen allergenic extract was conducted in 25 allergic subjects sensitized to short ragweed pollen. The objective of the study was to (1) identify the side-effects experienced, (2) estimate patients' tolerance to increasing doses and (3) evaluate the safety and compliance of self-administered doses. An escalating dosing course was employed to determine the maximal tolerable dose (MTD) for each subject. Based on the MTD and recorded responses, a safe starting dose was estimated and used to initiate an 8-week treatment course. During the 8-week "treatment course" each subject self-administered a total of up to 56 daily doses. Dose adjustments, if warranted, were made at two-week intervals. When doses were increased, then the first dose was administered in the clinic and the subject was observed for at least 20-30 minutes. At the completion of the 8-week course, the maximal doses achieved, side effects experienced, and compliance was evaluated. The results of the safety and dosing study showed that daily dosing of up to 60 Amb a 1 Units of Greer® Standardized Short Ragweed Pollen Allergenic Extract by the sublingual-oral route was generally safe and without adverse reactions. No emergency treatment (e.g. epinephrine) was required and overall compliance to the treatment course was greater than 85%. Based on this information, we designed the study described herein to further evaluate the safety and effectiveness of sublingual-oral immunotherapy using Standardized Short Ragweed Pollen Allergenic Extract.

A Phase IIB multi-center, parallel, randomized, double-blind placebo-controlled dose-response trial was conducted in adults using Greer® Standardized Glycerinated Short Ragweed Pollen Allergenic Extract administered by the sublingual-oral route. The preliminary dosing and treatment regimens were essentially as described above for the Phase I/II study. Based on the results obtained from the Phase I/II safety and dosing trial, two fixed daily target maintenance doses (4.8 and 48 Units Amb a 1) and a placebo were investigated. The primary outcome measure was the change in the average daily rhinoconjunctivitis symptom scores for actively treated and placebo groups recorded in Subject diaries during the ragweed pollen season in the Fall of 2006. The secondary outcome measures included (1) the average daily rhinoconjunctivitis symptom scores between actively treated groups; (2) the average daily medication scores between actively

treated and placebo groups; and (3) the average daily asthma symptom scores for actively treated and placebo groups. On average, the treatment groups achieved a reduction of 0.67 (95% CI: -0.16 – 1.50) in total rhinoconjunctivitis symptom scores during the ragweed pollen season. Additionally, anti-allergy medication scores were reduced in both dose groups by an average of 0.59 (95% CI: -0.10 – 1.27) and statistical significance (at the 5% level) was achieved in the high dose group during the peak ragweed pollen season. The total number of AEs reported as well as the percentage of subjects reporting AEs did not differ among active and placebo groups. There was a tendency, however, toward AEs being attributed to SLIT as the dosage was increased. In addition, of the 6 subjects who withdrew due to AEs, 1 was from the placebo group and 5 were from the high dose SLIT group; and among the 32 severe AEs experienced, one (shortness of breath) was probably related to SLIT and 5 were possibly related to SLIT (headache, nausea, abdominal pain, generalized itching, and sigmoid diverticulitis). These safety outcomes are consistent and similar to those made in published placebo-controlled SLIT trials.

An important factor that may have reduced the statistical power of the Phase IIB trial to detect differences in the primary endpoint was that more than 90% of the participants possessed co-seasonal or perennial allergies caused by allergens other than ragweed pollen. Symptoms caused by these other allergens may have accounted for a significant part of their overall symptoms during the ragweed pollen season. Thus, the use of baseline-adjusted scores or an analysis of covariance (ANCOVA) may be warranted. A regression analysis showed a strong relationship between the pre-seasonal baseline symptom scores and the scores reported during the ragweed pollen season ($r^2 = 0.46$). Furthermore, the standard error was reduced such that a trial requiring 815 subjects for analysis of symptom scores would require 250 for ANCOVA. Because it is extremely difficult if not impossible to recruit subjects who are mono-sensitized to short ragweed, the pre-seasonal symptom-medication score will be used to adjust the seasonal score for the primary efficacy variable in this Phase III trial.

2 OBJECTIVES

2.1 Study Objectives

The primary objective of this study is to demonstrate that SLIT administered pre- and co-seasonally is efficacious in reducing rhinoconjunctivitis symptoms and the use of allergy medication (calculated as a combined symptom and medication score) during the entire short ragweed pollen season.

Secondary objectives of this study are to demonstrate that SLIT administered pre- and co-seasonally:

- Reduces the rhinoconjunctivitis symptoms and the use of allergy medication (calculated as a combined symptom and medication score) during the 3 peak weeks of the short ragweed pollen season.
- Reduces the rhinoconjunctivitis symptoms during the entire short ragweed pollen season.
- Reduces the rhinoconjunctivitis symptoms during the 3 peak weeks of short ragweed pollen season.

The safety objective is to demonstrate that SLIT is safe as determined by self-reported AE's, concomitant medication use, vital signs, clinical laboratory evaluations and changes in physical examinations.

An exploratory objective will be to examine whether there are increases in beneficial immunoglobulins of ragweed-specific IgG and IgG₄ as well as increases in IgE antibody levels in serum.

3 STUDY DESIGN

This study is a randomized, double-blind, placebo-controlled parallel treatment group trial in adults to assess the efficacy and safety of sublingual-oral immunotherapy (SLIT) in the treatment of allergic rhinoconjunctivitis caused by seasonal ragweed pollen. This Phase 3 trial will be conducted in approximately 418 patients (209 per treatment group) at approximately 25-30 centers. The trial will start in March 2011 (before the natural ragweed pollinating season) and will end at the completion of the final visit, occurring after the end of the 2011 ragweed pollen season (October 2011). The SLIT will use currently licensed Greer® Standardized Short Ragweed Pollen Allergenic Extract. The objective is to demonstrate that SLIT reduces rhinoconjunctivitis symptoms and relief medication use as compared to treatment with placebo.

The study will be conducted in five parts (see Protocol Flowchart and Section 6- Study Schedule): (1) Screening, (2) Randomization and preliminary dosing to determine MTD, (3) 8-16 weeks of pre-seasonal treatment course (4) co-seasonal treatment course through the ragweed pollen season, and (5) post-treatment assessments.

The pre-treatment screening will be conducted to select suitable subjects for the study, obtain informed consent and pre-study clinical samples. Subject inclusion and exclusion criteria are detailed in Section 4- Study Enrollment and Withdrawal.

Eligible subjects will be allocated into treatment arms using a blocked randomization scheme, stratified by site. Once randomized, the subjects will be scheduled for a preliminary dosing visit to occur approximately 8-16 weeks prior to the anticipated beginning of the ragweed pollen season (preliminary dosing is not to occur less than 8 week prior to the anticipated season). During this visit, the subjects will receive up to three incremental sublingual doses of Greer® Standardized Short Ragweed Pollen Allergenic Extract or Placebo at 15-minute intervals while observed by the clinic staff (see Dosing Tables 6.3.1 and 6.3.2). The dose will be increased to the maximum dose unless the subject experiences moderated symptoms defined as definite awareness of symptoms that are bothersome but tolerable. The maximal tolerable dose (MTD) will be estimated for each subject and the MTD will serve as the subject's starting SLIT dose.

The treatment course will consist of daily self-administered doses of Greer® Standardized Short Ragweed Pollen Allergenic Extract or Placebo and will be taken in the morning before eating food. Subjects will record daily AM/PM rhinoconjunctivitis symptoms, relief medication use, and asthma symptoms using an e-diary. Each subject will be seen at the clinic every 30 days ($\pm$ 7 days) during the treatment course to evaluate tolerance and compliance; monitor adverse events; obtain clinical samples; and to adjust the dose, if necessary.

At the completion of the study (i.e., end of the ragweed pollen season), a post-treatment visit (Visit 7) will be scheduled to obtain post-study clinical samples, review subject diaries and notebooks for completeness, and to discharge subjects from the study.

4 STUDY ENROLLMENT AND WITHDRAWAL

Approximately 418 Subjects (209 per treatment group) will be enrolled into the study to ensure a minimum of 376 eligible Subjects complete the study. The study will be conducted at approximately 27 sites in the United States and Canada and enrollment need not be evenly distributed across sites. All Subjects must take part in the consent process. The person obtaining consent must explain all elements of the informed consent to the Subject. Adequate time must be allowed for the Subject to ask questions and make a voluntary decision. No protocol-specific procedures, including screening, are performed until the Subject has signed and dated an institutional review board (IRB)/Independent Ethics Committee (IEC)-approved informed consent form. When a Subject meets all of the inclusion and none of the exclusion criteria, the Subject will be registered, assigned a study number, and randomized for treatment on Day 0.

4.1 Subject Inclusion Criteria

1. Adult ragweed-sensitive subjects with allergic rhinoconjunctivitis with or without mild intermittent asthma (defined as GINA Class I, refer to Attachment 2). Asthmatic subjects must demonstrate an FEV_1 or $PEF \geq 80\%$ predicted; PEF or FEV_1 variability $<20\%$ at baseline;
2. Subjects must be 18 to 55 years of age to be enrolled in this study;
3. Test Subjects will have a history of moderate to severe allergic rhinoconjunctivitis symptoms which would normally require anti-allergy medications attributable to ragweed pollen for a minimum of two years before study entry. Subjects who have experienced such moderate to severe symptoms but have not necessarily taken anti-allergy medication for whatever reason, may be included;
4. Subjects will have a sum of erythema skin prick test reaction to short ragweed pollen allergenic extract equivalent to or greater than 60 mm ($\sum E \geq 60\text{mm}$) at 15-20 minutes after application. Skin prick tests will be performed with Standardized Short Ragweed Pollen Allergenic Extract along with negative (saline) and positive (histamine) controls using a Greer® Pick® skin test device;
5. All female Subjects of child-bearing potential will be required to provide a urine sample for pregnancy testing that must be negative no greater than 48 hours prior to administering study drug and at study completion before being discharged from the study. Child-bearing potential is defined from the onset of menstruation to one year post menopause and the absence of surgical sterilization; Females of child bearing potential must agree to notify the study staff of any late menstrual period, so a urine pregnancy test can be performed. A reliable form of birth control should be used while participating in this study and for 2 months following the last dose of the study drug. Reliable forms of birth control include oral contraceptive (birth control pills), an injection every 3 months (Depo-Provera®), a contraceptive patch (Ortho Evra), an

IUD (intrauterine device) or if partner has had a vasectomy. Otherwise, subject should agree to use double-barrier contraception in the form of a condom with a diaphragm, contraceptive sponge or cervical cap, and spermicide.

All male subjects MUST agree to use a medically acceptable contraceptive (abstain from sexual intercourse or use a condom WITH spermicide) throughout the study and for at least thirty (30) days after the study is over.

6. Subjects must be planning to remain in the study area during the ragweed season portion of the trial (see exclusion criteria # 14);
7. Subjects must be trained on the proper use of the EpiPen® Auto-Injector, and sign the EpiPen® Auto-Injector Training Form before being allowed to enroll in the study
8. Subjects must be mentally and physically capable of self-administering oral drug and using the electronic diary.

4.2 Subject Exclusion Criteria

1. Subjects having a history of anaphylaxis, or history consistent with persistent or unstable asthma;
2. Subjects with a sum of erythema skin prick test reaction to short ragweed pollen allergenic extract less than 60 mm ($\Sigma E < 60\text{mm}$) at 15-20 minutes after application.
3. Subjects with rhinoconjunctivitis symptoms attributable to perennial allergens (e.g. mite, dog, cat) that overlap the ragweed season (patients are allowed to be polysensitized, i.e. positive skin prick test in the absence of symptoms);
4. Subjects with chronic sinusitis, unstable angina, significant arrhythmia, uncontrolled hypertension, or other chronic or immunological diseases that in the opinion of the investigator might interfere with the evaluation of the test drug or pose additional risk to the Subject;
5. Subjects having perennial or structurally related rhinitis or rhinitis medicamentosa (from excessive use of nasal decongestants) that will interfere with the evaluation of symptoms due to ragweed allergy;
6. Asthmatic subjects with an FEV_1 or PEF $\leq 80\%$ predicted; PEF or FEV_1 variability $>20\%$ at baseline with or without controller medication;
7. Subjects who have received an experimental drug in the 30 days prior to admission into this study or who plan to use an experimental drug during the study;
8. Subjects who have received anti-IgE medications (Xolair®), or similar compounds in the last 12 months;

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9. Subjects who have received ragweed allergen immunotherapy in the last 3 years prior to admission into this study, or who have failed a course of subcutaneous ragweed allergen immunotherapy;
 10. Subjects who are current users of inhaled, oral, intramuscular, or intravenous corticosteroids, tricyclic antidepressants, beta blocker (oral or topical); or MAO inhibitors;
 11. Subjects using beta-2 agonists more than twice a week unless being taken only prior to exercise;
 12. Subjects using medications that could induce adverse gastrointestinal reactions during the study. Subjects using such medications must prove stable with no side effects for at least three months prior to enrollment in the study;
 13. Subjects refusing to sign the EpiPen® Auto-Injector Training Form;
 14. Females who are pregnant or breast feeding;
 15. Subjects who plan to leave the study area for more than 2 consecutive weeks during the ragweed pollen season.
 16. Subjects with a positive skin prick test to cat and/or dog, and who either owns the pet(s) to which they are allergic or have regular contact with cats or dogs;
 17. Subjects who sleep during the day due to working third shift;
 18. Subjects unable to achieve dose #2 or higher during preliminary dosing will be excluded.

4.3 Prior Treatment

Reasonable efforts will be made to determine all relevant treatment received by the Subjects within 30 days before administration of the study drug. All relevant information, including start/stop date, and doses, must be recorded on the Subject's case report form (CRF). The name of the procedure or drug and other information required is to be included on the CRF.

4.4 Treatment Assignment Procedures

4.4.1 Screen Failures

Subjects who sign an informed consent form and who either fail to meet the inclusion and/or exclusion criteria are defined as screen failures. For all screen failures, the investigator is to maintain a screening log that documents the Subject number, Subject initials, and reason(s) for screen failure. A copy of the screening log should be retained in the investigator's study files. Screen failures will not be included in any statistical analysis of the study data.

4.4.2 Randomization Procedures

All Subjects that are determined to be eligible for enrollment in the study will be allocated on a 1-to-1 basis to placebo or SLIT arms using a blocked randomization scheme by site.

4.4.3 Subject Identification and Masking Procedures

Each Subject will be assigned a unique identification number at screening that is retained for the duration of the study. Some Subjects will fail screening, but their identification numbers will not be reassigned or reused for any reason. Subjects who qualify for entry into the double-blind portion of the study will also receive a unique randomization number that links the Subject to the assigned (blinded) treatment.

The SLIT treatments will be supplied as 5-vial kits that contain one 20 mL vial for each of the 3 dose levels (see Section 6.3) plus two extra dose #3 vials used for re-supplying the Subject with study drug. One kit will be supplied for each randomized Subject. Each kit and each dose within the kit will contain unique identification numbers. The different doses within a kit will differ slightly in appearance, in that the color of the 140 µl actuator will be white, while the 50 µl actuator will be blue. However, the appearance of corresponding vials for SLIT and placebo groups will be identical in appearance and taste. Thus, dose level will be unblinded, but treatment (SLIT or placebo) will be blinded to Subjects and investigative site staff.

In the event of an SAE in which the treatment code needs to be broken (not all SAE's require the code be broken) for a specific study Subject, Investigators will have 24-hour access to treatment codes via a telephone number to Drug Safety. Drug Safety along with the attending physician will determine if the code needs to be broken. The study Medical Monitor will be the individual who is assigned the method to break the blind, inform the investigator or attending physician and complete the requisite code breaking CRF for the specific study Subject. The study Medical Monitor must notify the Sponsor's Project Manager within 24 hours of the code being broken for a particular subject.

4.4.4 Reasons for Withdrawal

Subjects can voluntarily withdraw from the study at anytime. Subjects can be withdrawn from the study at any time for any reason, including serious adverse events including, but not limited to:

1. Acute severe asthmatic reaction;
2. Anaphylactic reaction such as generalized angioedema, hypotension or arrhythmia;
3. Inability to tolerate the (procedures) due to excessive discomfort (i.e., sneezing, itchy mouth and/or ears, throat irritation, or cough);
4. Severe nausea, vomiting, diarrhea, or cramps;

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5. Development of any medical illness that is clearly not related to this protocol.

Follow-up information will be obtained for Subjects who discontinue their participation in the study. All procedures scheduled during the last Study Visit (Visit 7) should be completed for early termination Subjects (See the flowchart, Attachment 2 for procedures to be performed at the early termination visit.).

An investigator may terminate a Subject if s/he deems it clinically appropriate for any of the following reasons:

1. significant side effects from study drug;
2. serious or unexpected AEs;
3. failure to comply with the study protocol;
4. violations of eligibility criteria;
5. serious intercurrent illness;
6. administration of wrong lot of study drug;
7. progression of disease that requires alternative treatment.

4.4.5 Handling of Withdrawals

Follow-up information will be obtained for Subjects who are withdrawn from the study. No additional study drug/product will be given to any terminated or voluntarily withdrawing Subject.

Subjects who are terminated or voluntarily withdraw before Visit 7 will be asked to return for safety assessments. The following procedures should be conducted at termination; report of any adverse events, a complete physical examination, concomitant medications taken and any other study related tests needed. Subjects recording only mild or no symptoms at the last office visit will be discharged from the study. Subjects recording moderate or severe symptoms at the last office visit will require a follow-up every two weeks until symptom resolution, after which they will be discharged from the study (Case Report Form, Post-Treatment Visit). After the Subject's termination visit, the Subject will be followed for unresolved adverse events until they resolve. This will be done by phone contact with the Subject and recorded on the appropriate CRF.

For Subjects withdrawing from the study during a scheduled visit; the CRF for that visit will be completed along with the post-treatment and required procedures. Subject requesting withdrawal prior to their next scheduled visit will be required to come into the office for completion of the post-treatment visit. Subjects refusing to come back to the office for the post-treatment visit will require the site to make every effort to contact the Subject and collect study drug and the electronic diary. These attempts to contact the Subject must be

documented and a certified letter must be sent and filed in the CRF prior to considering the Subject lost to follow up.

Subjects withdrawn from the study will not be replaced, regardless of the reason for withdrawal.

It is important that every effort must be made to determine why a Subject fails to return for the necessary visits or is dropped from the study. This information should be documented and recorded on the Subject's CRF.

A Subject may be withdrawn from the study for not complying with the treatment regimen or the recording of symptoms and medication use. The expected compliance level will be 80% (diary completion/transmission, and study drug use compliance will be calculated via PHT StudyWorks). Subjects dropping below 80% will be re-educated by the site on the importance of diary completion and transmission. Compliance of less than or equal to 50%, after attempts of re-education, may be withdrawn from the study due to non-compliance. If the Subject's non-compliance is due to problems or errors with the electronic diary, then every effort must be made to trouble shoot and eliminate the problem with the electronic diary.

Subjects becoming pregnant during the study will stop taking study drug immediately and be withdrawn from the study. Subjects will be followed up throughout the pregnancy, delivery and examination of the infant for any congenital defects. All information will be documented on the Pregnancy Form.

5 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

5.1 Study Product Description

Greer® Standardized Short Ragweed Pollen Allergenic Extract along with placebo is formulated in 50% glycerococca's and contains 0.2% phenol.

5.1.1 Formulation, Packaging, and Labeling

Study product to be administered via the sublingual-oral route will be packaged in a 20 mL vial with a 140 µl (color white) or a 50 µl pump (color blue) applied as the closure along with the dispensing actuator. Each kit will have a number which is synonymous with the randomization number. The kit will have 5 vials that contain one 20 mL vial for each of the 3 dose levels (see Section 6.3) plus two extra dose #3 vials used for re-supplying the Subject with study drug. The vial will be labeled to comply with applicable local and international requirements and, at a minimum, will include the following information:

1. Blinding code (e.g. 001-02, in which 001 is the kit number while 02 is the vial number)
2. Protocol number
3. Mfg Date
4. IND statement.

5.1.2 Product Storage and Stability

The study product will be stored by the site and the study subject refrigerated at 2-8° C. Glycerinated, Standardized Short Ragweed Pollen Allergenic Extract has been shown to be stable for at least 12 months when stored refrigerated (2-8°C) and at room temperature (20-25°C) in stability studies using CBER/FDA approved potency assays. Greer® Glycerinated Standardized Short Ragweed Pollen Allergenic Extract packaged in the container-closure system will have stability monitored for at least the length of the trial.

5.2 Preparation and Administration of Study Product

Standardized Short Ragweed Pollen Allergenic Extract and Placebo will be manufactured and packaged in ready-to-use form by Greer® Laboratories under GMP and Greer Product License. The products will be stored at 2-8°C at the site until given to the study Subject at Visit 1 for administration with instructions for use.

5.3 Assessment of Subject Compliance with Study Intervention/Investigational Product

Study product will be first administered for training purposes and then dispensed to study subjects by qualified site personnel and compliance will be documented in the appropriate source documents and drug accountability records. Any compliance issues will be documented in the source document and on the drug inventory record. In addition, Greer® must be notified of any compliance issues.

5.4 Concomitant/Prior Medications

5.4.1 Concomitant Medications

Therapy that is considered necessary for the patient's welfare and will not interfere with the evaluation of the study treatments may be given at the discretion of the investigator.

Relief medication which has been provided should be used only on an as-needed basis to relieve moderate to severe nasal, conjunctival or asthma symptoms that patients are unable to tolerate.

5.4.2 Relief Medications

At the completion of Preliminary Dosing (Visit 1), Subjects will be allowed to take loratadine (10 mg tablets) and either Patanol® (twice daily preparations) or Pataday® (once daily preparation), on an as-needed basis to relieve moderate to severe nasal or conjunctival symptoms. Loratadine 10 mg tablets and Patanol®/Pataday® will be supplied by the sponsor. Asthmatic subjects will be permitted to use their own inhalers to administer beta-agonist should they experience asthma symptoms at any time during the study. The use of any relief medication will be fully recorded in the CRF. Analysis on the use of relief medications is further described in Section 12. An Epi-Pen® Auto-Injector will be provided to all Subjects and should only be used in the case of severe, systemic allergic reactions.

Relief Medication Use:

Patients will be provided with allergy relief medication and instructed, as follows, with respect to the use of these "rescue" medication to relieve breakthrough nasal or conjunctival (ocular) symptoms that are moderate to severe in intensity:

a) Patients are allowed, and will be instructed to use the oral antihistamine, Claritin® (loratadine; 10 mg tablet) with a daily dose of 1 tablet, on an as-needed basis to relieve moderate to severe nasal or ocular symptoms..

- The patient should be instructed to only use this oral medication for truly bothersome symptoms that are judged to be at least moderate if not severe in intensity.

b) Patients are allowed, and will be instructed to use an ocular antihistamine, Pataday® (olopatadine once daily preparation) or Patanol® (olopatadine, twice daily preparation) in each eye, only on an as-needed basis to relieve moderate to severe ocular symptoms

- The patient should be instructed to only use this ocular medication for truly bothersome eye symptoms that are judged to be at least moderate if not severe (and which did not respond to loratadine tablets).

c) Patients will be allowed to use albuterol (1-2 puffs, as-needed; up to 4 times a day) to relieve episodic asthma symptoms

- The patient should be instructed to only use this medication on an as-needed basis to relieve episodic chest tightness, cough, wheeze, and/or shortness of breath.

5.4.3 Prior Medications

The medications and their hold times to be observed prior to screening skin testing are listed in Attachment 1. Topical, oral or nasal spray corticosteroids will not be permitted at any time during the study, along with MAO inhibitors, tricyclic antidepressants, and beta blockers.

6 STUDY SCHEDULE

The study flow charts (Attachment 2) list the procedures to be performed at each visit. Each Subject must sign an IRB/Ethics committee approved informed consent form before any study procedures can take place. Once the informed consent is obtained, screening procedures can begin. Adverse events (AEs) must be recorded from the time the informed consent is signed. The investigator will maintain a master list of every Subject who has signed an informed consent form. A copy of the list will be retained in the investigator study files. The original signed informed consent must be kept with the individual Subject's records and a copy is to be given to the Subject.

The duration of the eligibility evaluation period will be determined by the amount of time needed to complete and evaluate the screening tests. It is expected that this evaluation period should be completed within 1 weeks; however, the period may be longer with the approval of the Medical Monitor. However, randomization and dosing must begin at least 8-16 weeks prior to the start of the typical ragweed season for each site, based upon historical pollen count records.

The study will be conducted in four parts:

1. Screening;
2. Randomization and Preliminary Dosing;
3. Treatment course;
4. Post-treatment visit.

6.1 Screening

The purpose of this visit is to evaluate the Subject to determine if they are eligible for enrollment. The informed consent agreement must be signed and dated before any of the following screening procedures can be performed:

- A medical history will be taken and a physical examination will be performed to verify the Subject's suitability for inclusion in the study. General demographic information (age, gender, etc.) will be captured on the Case Report Form.
- A urine hCG pregnancy test will also be performed on all female Subjects of child bearing potential 48 hours prior to administering study drug as defined in the Inclusion Criteria of this protocol.
- A pulmonary function test will be administered to asthmatic Subjects to verify the Subject's suitability for inclusion in the study (see Section 4.1 and 4.2).
- Subjects will receive prick tests with Greer® standardized short ragweed pollen, house dust mite, cat hair, grass pollen, *Alternaria*, dog epithelia, and lamb's quarter

pollen allergenic extracts along with histamine and diluent controls to confirm sensitivity to the relevant allergen.

- Two 10 mL tubes of whole blood with no preservative or gel barrier tube will be collected by venipuncture, clotted, centrifuged and the serum aliquoted into 1 plastic transfer tube for chemistry and 1 cryogenic transfer vial for allergen-specific antibody testing.
- One 3.0 to 5.0 ml EDTA tube will also be collected and sent to the Central Lab for CBC with Differential. Differential counts are automated on the Central lab's hematology analyzer so there is no need to prepare slides. Blood chemistry results must be obtained from the Central Lab prior to proceeding with preliminary dosing.

6.2 Stratification, Randomization and Allocation of Subjects

All Subjects determined to be eligible for randomization in the study will be allocated into treatment groups using a blocked randomization scheme that is stratified by site. A computer based randomization method will be used to prepare two separate randomization lists. Subjects will sequentially be assigned the next available randomization number. Once allocated, the subjects will be scheduled for a preliminary dosing visit. This visit should occur no less than approximately 8 weeks prior to the anticipated beginning of the ragweed pollen season.

6.3 Preliminary Dosing Visit 1

During the preliminary dosing visit, the Subjects will be randomized and then receive up to three incremental doses of Greer® Standardized Glycerinated Short Ragweed Pollen Allergenic Extract or Placebo (see dose tables below) at 15-20 minute intervals while observed by the clinic staff. The doses will be increased until the Subject experiences discomfort defined as definite awareness of a sign/symptom that is bothersome, but tolerable (moderate).

During the visit, the principal investigator or sub-investigator will see each Subject to assess their fitness to continue and to review any side-effects or adverse reactions experienced by the test Subjects. Additionally, the lung function of asthmatic Subjects will be monitored after each preliminary dose via a peak flow meter and evaluated for the continuation of preliminary dosing.

The investigator/designate will administer the doses and any relief medications, as required.

The test Subjects will report symptoms using the electronic diaries 15 minutes after each dose is administered in order to identify and grade symptoms.

Dose escalation will continue according to the schedule until the maximum tolerable dose (MTD), defined as the definite awareness of a sign/symptom that is bothersome, but tolerable (moderate symptom) or until the maximum dose on the escalating dose table is

reached, whichever occurs first. The lower of the MTD or the maximum dose will be the dose that is sent home with the Subject for once-daily self-administration during the study. Subjects not achieving dose #2 or higher will not be allowed to continue in the study.

6.3.1 Escalating Dosing Table: SLIT Group

Dose #	Concentration ¹	Volume	Dose
1	0 Units Amb a 1/mL	140 µL	0 Units Amb a 1
2	300 Units Amb a 1/mL	50 µL	15 Units Amb a 1
3	300 Units Amb a 1/mL	140 µL	42 Units Amb a 1

6.3.2 Escalating Dosing Table: Placebo Group

Dose #	Concentration ¹	Volume	Dose
1	0 Units Amb a 1/mL	140 µL	0 Units Amb a 1
2	0 Units Amb a 1/mL	50 µL	0 Units Amb a 1
3	0 Units Amb a 1/mL	140 µL	0 Units Amb a 1

¹ Concentrations and doses are presented in terms of Units of Amb a 1 determined by radial immunodiffusion assay using CBER/FDA reference reagents as described in the CBER/FDA Manual of Methods and Greer's STN101844.

Subjects will receive a vial of the (blinded) treatment, one EpiPen® Auto-Injector, one electronic diary, and a Subject Notebook. Loratadine 10 mg tablets and olopatadine eye drops will be supplied as describe in Section 5.5.2. Subjects will be given written instructions on the proper use, storage and administration of the extract prior to initiating the course. Subjects must sign the EpiPen® Auto-Injector training form and understand its use before being sent home with study drug. Subjects will also be trained on how to enter their symptoms into the electronic diary and how to transmit those data to a central database. Subjects are required to bring their study drugs, relief medications, electronic diary and Subject Notebook with them to every visit.

6.4 Treatment Course

The treatment course will consist of daily self-administered doses of Greer® Standardized Short Ragweed Pollen Allergenic Extract or Placebo starting approximately 16 weeks and no later than 8 weeks prior to the historical start of ragweed pollen season (Ragweed season typically begins around the 1st or 2nd week in August). This may vary from site to site based on geographical location), and ending after the ragweed pollen season in October 2011. Each Subject will be required to return to the study site every four weeks to evaluate tolerance, compliance, and to incrementally adjust the dose as deemed appropriate.

Subjects will record daily symptoms and anti-allergy/asthma medication use in the LogPad® electronic diary. The Subjects will be instructed to immediately contact the site to report any adverse event or to seek medical advice.

Subjects will be seen every 30 days ($\pm$ 7 days) at the study site during the treatment phase of the study. Subjects will not take their morning dose of extract on days they are scheduled to come into the office. During these visits, the principal investigator or sub-investigator will review the symptom diaries and notebook entries, and determine whether up/down dose adjustments should be made. Decreasing a Subject to dose level #1 will not be allowed at any time during the study (see section 7.1.4 for details).

Subjects will take their dose, adjusted or not, at the conclusion of the exam. These visits should be scheduled no later than 12:30 P.M. in order for the Subject to meet the 10-14 hour window for recording symptoms in the electronic diary.

Subject notebooks are to be used to record concomitant medications that have been taken (other than relief medications, Section 5.5.2) and adverse events other than those recorded in the electronic diary.

During these visits, Subjects will bring their treatment vials for inspection to confirm adherence to the treatment regimen.

At the end of each site's 2011 ragweed pollen season, each site will contact their subjects and inform them to stop their drug treatment regimen, and schedule the Post Treatment Visit.

6.5 Post Treatment Visit

A post-study visit will be conducted at the conclusion of the treatment course, including early terminations.

A physical exam will be performed.

Two 10 mL tubes of whole blood with no anticoagulant or gel barrier tubes will be collected by venipuncture, clotted, centrifuged and the serum aliquoted into 1 plastic transfer tube for chemistry and 1 cryogenic transfer vial for allergen-specific antibody testing.

One 3.0 to 5.0 ml EDTA tube will also be collected and sent to the Central Lab for CBC with Differential.

A urine hCG pregnancy test will also be performed on all female Subjects of child bearing potential prior to study discharge.

Electronic diaries, subject notebooks and study medications must be collected at this visit.

The investigator/designate will review the symptom diaries and notebook entries to ensure they are complete and no clarification is needed.

7 STUDY PROCEDURES/EVALUATIONS

7.1 Clinical Evaluations

7.1.1 Medical History

A medical history associated with the Subject's skin, head, eyes, nose and throat, respiratory, cardiovascular, gastrointestinal, genitourinary, neurological, blood/lymphatic, musculoskeletal systems, psychological/psychiatric and allergic conditions will be obtained by interview and/or from medical records. Conformance with the inclusion/exclusion criteria will be confirmed.

7.1.2 Medications History

If any prescription or over-the-counter medications are currently being taken by the Subject, they will be documented. Assessment of eligibility should include a review of permitted and prohibited medications.

7.1.3 Physical Exam

Physical examination will consist of an assessment of the Subject's general appearance, skin, head and neck, lymph nodes, thyroid, musculoskeletal system and extremities, cardiovascular system, lungs, abdomen, neurological condition. Demographic information such as age, gender, race, height and weight will be recorded along with vital signs that include heart rate, blood pressure and temperature.

7.1.4 Skin Prick Test

Skin prick tests will be performed on the volar surface of the arms using the Greer® Pick® with standardized short ragweed pollen, house dust mite, cat hair, timothy grass pollen, *Alternaria*, dog epithelia, and lamb's quarter pollen allergenic extracts along with histamine and diluent controls to confirm sensitivity to the relevant allergen. The prick tests will be read at 15-20 minutes following application. A positive test will be defined as a sum erythema equivalent to or greater than 60 mm ($\sum E \geq 60\text{mm}$). The wheal and erythema reactions will be outlined with a ball point pen and transferred with transparent tape to the data collection form. The reaction diameter is defined as one-half the sum of largest diameter and the midpoint orthogonal diameter wheal or erythema.

7.1.5 Criteria for Dose Adjustments

1. If the Subject reports any severe symptoms during the treatment course, a dose reduction will be considered by the Investigator..
2. If the Subject reports no severe symptoms, then a dose increase will be considered by the Investigator.

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3. Increases to a Subject's dose will be done at scheduled office visits. A decrease in a Subject's dose may warrant the subject to come into the office prior to their scheduled visit in order to alleviate symptoms caused by the higher dose and promote compliance. Unscheduled office visits such as one needed to decrease the dose will be recorded on the unscheduled visit CRF. An unscheduled visit does not take the place of a scheduled visit and the Subject would be expected to return to the clinic for their regularly scheduled visit.
 4. Decreasing a Subject to dose level #1 will not be allowed at anytime during the study. Investigators may temporarily stop the administration of study drug for Subjects experiencing severe symptoms while on dose level # 2 for no greater than 7 days. At the end of the 7 days the Subject will begin to re-administer dose level #2. Subjects may be withdrawn from the study if severe symptoms persist or re-appear and they are unable to tolerate dose level # 2.
 5. When adjusting a Subject's dose (up or down) the coordinator will collect the vial which the subject was administering and place it back in the specific subject kit kept at the study site, while removing the new dose vial from the kit and giving it to the Subject (e.g. Subject is on dose 2 and has recorded no severe symptoms since previous visit; the subject's dose would be increased to dose 3. Likewise, if a Subject was on dose 3 and was recording severe symptoms they will be brought into the office and given dose 2).
 6. Dose adjustments will be recorded on the appropriate CRF page.

7.1.6 Review of Symptom Scores

Symptom scores will be reviewed at least every 72 hours by the clinical coordinator at each study site to assess Subject's compliance and to monitor the occurrence of severe symptoms. If compliance issues are identified as per study staff, the Subject will be called for resolution.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

1. Pregnancy testing will be conducted at the screening visit and upon completion of the study using an FDA-approved urine hCG pregnancy test. Additional testing may be requested when female subjects report missing their menstrual period.
2. Urinalysis and General blood chemistry testing will be performed by a CLIA certified laboratory, which includes CBC with Differential, ALT (SGPT), AST (SGOT), Creatinine, Glucose, Potassium, Sodium, and Urea Nitrogen. Subjects with urinalysis and/or blood chemistry results falling outside the normal ranges will be evaluated for continuation in the study and may be withdrawn at the discretion of the investigator..

7.2.2 Ragweed Pollen Counts

Natural exposure to ragweed pollen from March to October will be estimated on a weekly basis for each study site using standardized methods described by the AAAAI Aerobiology Committee. The average weekly ragweed pollen count (grains/m³) will be based on samples collected using a Burkard volumetric spore trap, a Kramer-Collins sampler or a Rotorod sampler, which are appropriately situated for airborne pollen sampling. The official start of ragweed season is defined as the first day in which ragweed pollen counts are >10 grains/m³. The official end of ragweed season is defined as the first of the three consecutive days on which ragweed pollen counts are < 5 grains/m³. In the event that, after these three days the pollen count is greater than 5 grains/m³ for three consecutive days then the season will be considered resumed and the former dates considered a season interruption. All of these days including the interruption period will then be considered part of the entire ragweed season. Spring and summer pollen counts (trees and grass) will also be collected at each site.

Sites are expected to have pollen counting conducted/provided for a minimum of three days per week and preferably 5 days per week. The exception to this would be days that counting was not conducted due to rain.

7.2.3 Special Assays or Procedures

Laboratory assays will be performed for ragweed-specific serum IgE, IgG and IgG₄ measured pre- and post-treatment.

8 ASSESSMENT OF SAFETY

8.1 Contraindications, Precautions, and Warnings

Greer® Standardized Short Ragweed Pollen Allergenic Extract is supplied ready to use via the sublingual-oral route. Allergenic Extracts are manufactured to assure high potency and have the ability during sublingual-oral administration to cause serious local and systemic reactions in sensitive subjects. Most reactions occur within 20 minutes after dosing [28], but may occur later [29]. To minimize the potential for local or systemic reactions, the relative sensitivity of the subject must be assessed from the allergic history and from clinical observations. Subjects should be informed of these risks prior to undergoing dosing via the sublingual route (see PRECAUTIONS and ADVERSE REACTIONS below).

The physician must be prepared to treat anaphylaxis should it occur and have the necessary drugs and equipment on hand to do so [30].

Subjects receiving Allergenic Extracts should be kept under observation a minimum of thirty minutes so that any adverse reaction can be observed and handled properly.

Systemic adverse reactions may occur within minutes upon use of an Allergenic Extract to which a person has specific sensitivity. The safety profile reported in studies from Europe [19-24] show the most frequently reported side effects was oral itching followed by gastrointestinal complaints. Oral itching was always reported as mild and self-resolving. Gastrointestinal complaints were associated with doses greater than 100 times that given during subcutaneous immunotherapy. Rhinitis, conjunctivitis, tiredness, and urticaria have been reported less frequently and their incidence (0.01-0.05%, or 1 to 5 per 10,000 doses) did not differ from the placebo group. Our double-blind, placebo-controlled SLIT trials have been consistent with these published studies and showed that SLIT was well-tolerated. The incidence of treatment emergent adverse events (TEAEs) was comparable between active and placebo-treated groups: 43.5% vs. 45.8% in the SLIT- and placebo-treated groups, respectively. The most common TEAEs were sinusitis, headache, throat irritation in SLIT-treated subjects; and headache, sinusitis, and upper respiratory tract infection in placebo-treated subjects. In comparison, systemic adverse events with subcutaneous immunotherapy range between 0.1 to 1% (10 to 100 per 10,000 doses) of which 5-10% may be severe [25-27]. Thus, the safety profile of sublingual immunotherapy is expected to be superior to the subcutaneous route even when significantly higher doses of allergenic extract are administered.

A review of articles on SLIT indicates that severe adverse reactions including anaphylaxis are very rare, and no fatalities have been reported. Two anaphylactic events occurred on the first dose of a grass tablet in patients who had previously had systemic reactions with SCIT. Two occurred in patients who received multi-allergen SLIT. No reported anaphylactic reactions to SLIT short ragweed have occurred [31-35].

8.2 Specification of Safety Parameters

Adverse events or experiences will be described, investigated and reported according to procedures outlined in Section 8.3.2 Recording and Reporting Adverse Events. The study will be performed at sites equipped to deal with any emergency adverse reactions to the allergenic extracts. Because there is a possibility that immediate or delayed allergic reactions may occur, physicians and technicians conducting the studies will be experienced in the treatment of severe systemic reactions.

8.2.1 Definitions

An **adverse event (AE)** is any untoward, undesired, unplanned clinical event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a human being participating in a clinical study with a Sponsor study drug, regardless of causal relationship. An adverse event may include:

1. Any clinically significant worsening of a preexisting condition;
2. Any recurrence of a preexisting condition;
3. An AE occurring from overdose of a Sponsor study drug whether accidental or intentional (i.e., a dose higher than that prescribed by a health care professional for clinical reasons);
4. An AE occurring from abuse of a Sponsor study drug (i.e., use for non-clinical reasons);
5. An AE that has been associated with the discontinuation of the use of a Sponsor study drug.

A **preexisting condition** is a clinical condition (including a condition being treated) that is diagnosed before the Subject signs the informed consent form and that is documented as part of the Subject's medical history.

An AE is considered to be **treatment emergent** if (a) it was not present when the active phase of the study began and is not a chronic condition that is part of the Subject's medical history, or (b) it was present at the start of the active phase of the study or as part of the Subject's medical history, but the severity or frequency increased during the active phase.

The **active phase of the study** begins at the time of the first dose of the study.

A **serious adverse event (SAE)** is any AE occurring that results in 1 or more of the following outcomes:

1. Death;

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2. Life threatening situation (see below);
 3. In-patient hospitalization or prolongation of an existing hospitalization (see below);
 4. Persistent or significant disability or incapacity (see below);
 5. Congenital anomaly or birth defect.

Additionally, important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the Subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not require hospitalization, or development of drug dependency or drug abuse. Note: If there is any doubt whether the information constitutes an adverse event, the information will be treated as an adverse event.

A **life threatening adverse event** is any AE that places the Subject at immediate risk of death from the event as it occurred. A life-threatening event does not include an event that might have caused death had it occurred in a more severe form but that did not create an immediate risk of death as it actually occurred. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening, even though drug-induced hepatitis of a more severe nature can be fatal.

Hospitalization is to be considered only as an overnight admission. Hospitalization or prolongation of a hospitalization is a criterion for considering an AE to be serious. In the absence of an AE, the participating investigator should not report hospitalization or prolongation of hospitalization on a form. In addition, a hospitalization planned before the start of the study for a preexisting condition that has not worsened does not constitute an SAE (e.g., elective hospitalization for a total knee replacement due to a preexisting condition of osteoarthritis of the knee that has not worsened during the study).

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

8.2.2 Adverse Events, Expected Risks and Discomforts

Examples of adverse events (defined as any untoward medical occurrence in a Subject administered a pharmaceutical/biological product and which *does not necessarily have to have a causal relationship with this treatment*) include but are not limited to:

1. Eye symptoms (itchy, runny, swelling);
2. Nose symptoms (sneezing, itching, runny stuffy);
3. Mouth/ears/throat symptoms (itchy mouth, throat irritation, cough, itchy ears);

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4. Skin symptoms (angioedema and/or urticaria generalized pruritis);
 5. Gastrointestinal symptoms (vomiting, diarrhea, cramps, nausea);
 6. Lung symptoms (wheezing, coughing shortness of breath);
 7. Other. If “other” is checked, a specific description of signs or symptoms will be listed.

Examples of Serious Adverse Events include but are not limited to

1. Unconsciousness;
2. Shock;
3. Drop in blood pressure;
4. Lower airway obstruction;
5. Upper airway obstruction;
6. Other. If “other” is checked, a specific description of signs or symptoms will be listed.

Discomfort caused by sublingual-oral administration of allergenic extracts is believed to be low based on the experience in many allergy clinics where this modality of treatment has been routinely performed. The Subject may experience some discomfort up to a few days at the site of the skin test, nasal provocation test, or blood draw. This is usually self-limited and does not require further treatment.

All Subjects will be instructed to contact the clinic to report any adverse reactions experienced during the course of the study.

8.3 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters (Adverse Events)

Adverse events or experiences and those events that lead to discontinuation of treatment will be recorded and monitored. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate 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 occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

8.3.1 Reportable Events/Information

An AE or SAE that occurs between the times that the Subject signs the informed consent form through 30 days from the Subject's last dose, regardless of study drug or protocol relationship should be reported. This includes events that emerge during the screening and pretreatment run-in periods. All AEs and SAEs will be recorded on source documents and recorded on CRFs. All AEs and SAEs that occur after the screening period will also be recorded on the CRFs. The Drug Safety Medical Monitor must instruct the investigator to follow up as is medically necessary on all AEs, SAEs, and other reportable events until the event has subsided or values have returned to baseline, or in case of permanent impairment, until the condition stabilizes.

The investigator/designate will provide all documentation pertaining to the event (e.g., additional laboratory tests, consultation reports, discharge summaries, postmortem reports, etc.) to the Drug Safety monitor in a timely manner. Reports relative to the Subject's subsequent course must be submitted to Drug Safety until the event has subsided or, in case of permanent impairment, until the condition stabilizes.

The following events will be recorded and reported in the same time frame and following the same process as for SAEs:

1. Inadvertent or accidental exposure to study drug with or without an AE.
2. SAEs occurring after unauthorized or accidental use in persons not participating in the study.
3. Abnormal biological or vital signs values that are considered clinically relevant by the participating investigator. These must be reported in the same time frame and following the same process as for an AE or an SAE.
4. Study product abuse and overdose. Any dose of study medication greater than the dose prescribed by the protocol at any given time period will be considered an overdose. Regardless of whether the overdose is intentional or accidental, it is a reportable event and Drug Safety must be notified immediately. A detailed description of the treatment(s) administered for the overdose and the Subject's clinical course will be recorded on the CRFs provided. The physician managing the overdose may order any additional test(s) he or she thinks is necessary to manage the Subject properly.

8.3.2 Recording and Reporting Adverse Events

At each required study visit, all AEs that have occurred since the previous visit must be documented and recorded in the adverse event record of the Subject's CRF. The information recorded should be based on the signs or symptoms detected during the physical examination and clinical evaluation of the Subject. In addition to the information obtained from those sources, the Subject should be asked the following nonspecific question: "How have you been feeling since your last visit?" Signs and symptoms should be recorded using standard medical terminology.

The following AE information must be included (when applicable): the specific condition or event and direction of change; whether the condition was preexisting (i.e., an acute condition present at the start of the study or history of a chronic condition) and, if so, whether it has worsened (e.g., in severity and/or frequency); the dates and times of occurrence; severity; causal relationship to study drug; action taken; and outcome.

When assessing the relationship between administration of a study drug and an AE, the following should be considered:

1. Temporal relationship between administration of the study drug and the AE.
2. Biological plausibility of relationship.
3. Subject's underlying clinical state or concomitant agents and/or therapies.
4. When applicable, whether the AE abates on discontinuation of the study product.
5. When applicable, whether the AE reappears on repeat exposure to the study product.

SAEs that are not study product related may nevertheless be considered by the participating investigator or the Medical Monitor (or designee) to be related to the conduct of the clinical study, i.e., to a Subject's participation in the study. For example, a protocol-related SAE may be an event that occurs during a washout period or that is related to a procedure required by the protocol.

8.3.3 Timing for Reporting Adverse Events

Any SAE, regardless of causal relationship, must be reported to Drug Safety immediately. A completed Serious Adverse Event Form must be faxed to the number indicated at the front of this protocol no later than 24 hours after the investigator/designate becomes aware of the SAE. A phone call should be made to confirm that the SAE fax was received. Compliance with this time requirement is essential for Drug Safety to comply with regulatory obligations.

Follow-up information relating to an SAE must be reported to Drug Safety within 24 hours of receipt by the investigator by faxing a completed serious adverse event form to the number indicated in the front of this protocol and confirming by telephone that the fax was received. The Subject should be observed and monitored carefully until the condition resolves or stabilizes or its cause is identified.

Any emergency must be reported to Drug Safety immediately (within 24 hours) by contacting Nadine Urac listed in the front of this protocol.

In the event a treatment code needs to be broken, contact Drug Safety at the phone number listed in the front of this protocol (see section 4.4.3).

For all other inquiries and information about this study, contact your study monitor.

8.3.3.1 Severity of Event:

The following definitions should be used when determining the severity of an AE. Please note that there are some cases in which removal of study drug may be appropriate.

1. Mild (grade 1): The AE is noticeable to the Subject but does not interfere with routine activity. The AE does not require discontinuing administration or reducing the dose of the study drug.
2. Moderate (grade 2): The AE interferes with routine activity but responds to symptomatic therapy or rest. The AE may require reducing the dose but not discontinuing administration of the study drug.
3. Severe (grade 3): The AE significantly limits the Subject's ability to perform routine activities despite symptomatic therapy. In addition, the AE leads to discontinuing administration or reducing the dose of the study drug.
4. Life Threatening (grade 4): The AE requires discontinuing administration of the study drug. The Subject is at immediate risk of death.

8.3.3.2 Relationship to Study Product:

The clinician's assessment of an AE's relationship to test article (vaccine or study drug) 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 product assessed using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

1. Definitely related: Event can be fully explained by administration of the study drug.

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2. Probably related: Event is most likely to be explained by administration of the study drug rather than the Subject's clinical state or other agents/therapies.
 3. Possibly related: Event may be explained by administration of the study drug or by the Subject's clinical state or other agents/therapies.
 4. Probably not related: Event is most likely to be explained by the Subject's clinical state or other agents/therapies, rather than the study drug.
 5. Definitely not related: Event can be fully explained by the Subject's clinical state or other agents/therapies.

8.3.4 Reporting of Pregnancy

Pregnancy will be reported using the Pregnancy Notification Form, and Subject will be withdrawn immediately.

8.4 Stopping Rules

The study will be stopped (meaning all administration of SLIT doses will be temporarily discontinued for all subjects at each site) if the same or similar serious adverse event occurs in at least two subjects in which the SAE was determined to be related to the study drug. Overall stopping rules include:

1. Death of an individual
2. Acute anaphylaxis in which rescue medication is administered
3. Significant drop in blood pressure: Systolic <90 mm Hg or <80% of baseline, whichever is higher, and Diastolic blood pressure <60 mmHg-absolute
4. Severe asthmatic exacerbation requiring hospitalization.
5. Significant cardiac arrhythmias

If the study is stopped due to meeting any of the above criteria, it may not be resumed until all pertinent information is discussed with the Medical Monitor and Sponsor, and there is agreement with the resumption of the study. All IRB/Ethics committees should be notified of the interruption or termination of the study and the reasons for those actions and the rationale for resuming the study, if that is the case. Subsequent review of serious, unexpected, and related AEs by the IEC/IRB, the sponsor, and the Medical Monitor or relevant regulatory authorities may also result in suspension of further trial interventions/administration of study product at a site. The Medical Monitor and study Sponsor retain the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

Additionally, the study will be stopped upon enrollment and completion of the defined number of Subjects that meet the selection criteria.

Statistical Analysis Plan

1. INTRODUCTION

This SAP is based on the final Protocol SLIT 10-01 [REDACTED]. The SAP provides details on the planned statistical methodology for analysis of the study data. The SAP also outlines the statistical programming specifications for the tables, listings and figures.

This SAP describes the study endpoints, derived variables, anticipated data transformations and manipulations, and other details of the analyses not provided in the study protocol. This SAP therefore outlines in detail all other aspects pertaining to the planned analyses and presentations for this study.

The following documents were reviewed in preparation of this SAP:

- Clinical Research Protocol SLIT 10-01 [REDACTED]
- Final Case Report Forms (CRFs) for protocol R10-1148/SAF (SLIT 10-01/Short Ragweed Pollen Extract) Template_v4.0 dated 5-Jul-2011

The reader of this SAP is encouraged to also read the clinical protocol for details on the conduct of this study, and the operational aspects of clinical assessments and timing for completing a subject in this study.

2. OBJECTIVES

Primary Objective:

The primary objective of this study is to demonstrate that SLIT administered pre- and co-seasonally is efficacious in reducing rhinoconjunctivitis symptoms and the use of allergy medication (calculated as a combined symptom and medication score) during the entire short ragweed pollen season.

Secondary Objectives:

Secondary objectives of this study are to demonstrate that SLIT administered pre- and co-seasonally:

- Reduces the rhinoconjunctivitis symptoms and the use of allergy medication (calculated as a combined symptom and medication score) during the 3 peak weeks of the short ragweed pollen season.
- Reduces the rhinoconjunctivitis symptoms during the entire short ragweed pollen season.
- Reduces the rhinoconjunctivitis symptoms during the 3 peak weeks of short ragweed pollen season.

Safety Objectives:

The safety objective is to demonstrate that SLIT is safe as determined by self-reported AE's, concomitant medication use, vital signs, clinical laboratory evaluations and changes in physical examinations.

Exploratory Objective

An exploratory objective will be to examine whether there are increases in beneficial immunoglobulins of ragweed-specific IgG and IgG4 as well as increases in IgE antibody levels in serum.

3. STUDY DESIGN

This study is a randomized, double-blind, placebo-controlled parallel treatment group trial in adults to assess the efficacy and safety of sublingual-oral immunotherapy (SLIT) in the treatment of allergic rhinoconjunctivitis caused by seasonal ragweed pollen. This Phase 3 trial will be conducted in approximately 418 patients (209 per treatment group) at approximately 25-30 centers. The trial will start in March 2011 (before the natural ragweed pollinating season) and will end at the completion of the final visit, occurring after the end of the 2011 ragweed pollen season (October 2011). The SLIT will use currently licensed Greer® Standardized Short Ragweed Pollen Allergenic Extract. The objective is to demonstrate that SLIT reduces rhinoconjunctivitis symptoms and relief medication use as compared to treatment with placebo.

The study will be conducted in five parts (see Protocol Flowchart and Section 6- Study Schedule): (1) Screening, (2) Randomization and preliminary dosing to determine MTD, (3) 8-16 weeks of pre-seasonal treatment course (4) co-seasonal treatment course through the ragweed pollen season, and (5) post-treatment assessments.

The pre-treatment screening will be conducted to select suitable subjects for the study, obtain informed consent and pre-study clinical samples. Subject inclusion and exclusion criteria are detailed in Section 4- Study Enrollment and Withdrawal.

Eligible subjects will be allocated into treatment arms using a blocked randomization scheme, stratified by site. Once randomized, the subjects will be scheduled for a preliminary dosing visit to occur approximately 8-16 weeks prior to the anticipated beginning of the ragweed pollen season (preliminary dosing is not to occur less than 8 week prior to the anticipated season). During this visit, the subjects will receive up to three incremental sublingual doses of Greer® Standardized Short Ragweed Pollen Allergenic Extract or Placebo at 15-minute intervals while observed by the clinic staff (see Dosing Tables 6.3.1 and 6.3.2). The dose will be increased to the maximum dose unless the subject experiences moderated symptoms defined as definite awareness of symptoms that are bothersome but tolerable. The maximal tolerable dose (MTD) will be estimated for each subject and the MTD will serve as the subject's starting SLIT dose.

The treatment course will consist of daily self-administered doses of Greer® Standardized Short Ragweed Pollen Allergenic Extract or Placebo and will be taken in the morning before eating food. Subjects will record daily AM/PM rhinoconjunctivitis symptoms, relief medication use, and asthma symptoms using an e-diary. Each subject will be seen at the clinic every 30 days (± 7 days) during the treatment course to evaluate tolerance and compliance; monitor adverse events; obtain clinical samples; and to adjust the dose, if necessary.

At the completion of the study (i.e., end of the ragweed pollen season), a post-treatment visit (Visit 7) will be scheduled to obtain post-study clinical samples, review subject diaries and notebooks for completeness, and to discharge subjects from the study.

4. STUDY PRODUCT DESCRIPTION

Greer® Standardized Short Ragweed Pollen Allergenic Extract along with placebo is formulated in 50% glycerococca's and contains 0.2% phenol.

5. SUBJECT IDENTIFICATION AND MASKING PROCEDURES

Each Subject will be assigned a unique identification number at screening that is retained for the duration of the study. Some Subjects will fail screening, but their identification numbers will not be reassigned or reused for any reason. Subjects who qualify for entry into the double-blind portion of the study will also receive a unique randomization number that links the Subject to the assigned (blinded) treatment.

The SLIT treatments will be supplied as 5-vial kits that contain one 20 mL vial for each of the 3 dose levels (see Section 6.3) plus two extra dose #3 vials used for re-supplying the Subject with study drug. One kit will be supplied for each randomized Subject. Each kit and each dose within the kit will contain unique identification numbers. The different doses within a kit will differ slightly in appearance, in that the color of the 140 μ L actuator will be white, while the 50 μ L actuator will be blue. However, the appearance of corresponding vials for SLIT and placebo groups will be identical in appearance and taste.

Thus, dose level will be unblinded, but treatment (SLIT or placebo) will be blinded to Subjects and investigative site staff.

In the event of an SAE in which the treatment code needs to be broken (not all SAE's require the code be broken) for a specific study Subject, Investigators will have 24-hour access to treatment codes via a telephone number to Cetcro Drug Safety. Drug Safety along with the attending physician will determine if the code needs to be broken. The study Medical Monitor will be the individual who is assigned the method to break the blind, inform the investigator or attending physician and complete the requisite code breaking CRF for the specific study Subject. The study Medical Monitor must notify the Sponsor's Project Manager within 24 hours of the code being broken for a particular subject.

6. STRATIFICATION, RANDOMIZATION AND ALLOCATION OF SUBJECTS

All Subjects determined to be eligible for randomization in the study will be allocated into treatment groups using a blocked randomization scheme that is stratified by site. A computer based randomization method will be used to prepare two separate randomization lists. Subjects will sequentially be assigned the next available randomization number. Once allocated, the subjects will be scheduled for a preliminary dosing visit. This visit should occur no less than approximately 8 weeks prior to the anticipated beginning of the ragweed pollen season.

All Subjects that are determined to be eligible for enrollment in the study will be allocated on a 1-to-1 basis to placebo or SLIT arms using a blocked randomization scheme by site.

7. SAMPLE SIZE CONSIDERATIONS

In the previous phase III double blind placebo control trial (2008-2009), the primary efficacy variable was the net average daily Rhinoconjunctivitis Symptom Score (netRSS) during the ragweed pollen season. In that trial, the SLIT group was not significantly better than the Placebo. To explore further, a post hoc subgroup analysis was performed on the data of that study.

The study patients were divided into two subsets of patients the using sum of erythema (ΣE) skin prick reaction at 15-20 minutes after application to short ragweed pollen allergenic extract as the divider. In the subset of patients with $\Sigma E \geq 60\text{mm}$, the SLIT group had significantly more reduction in netRSS comparing to the Placebo group with a 2-sided p-value of 0.01.

In that trial in patients with $\Sigma E \geq 60\text{mm}$, means and SDs of netCOMBSC in the two treatment groups were:

1. SLIT Group:	Mean = 2.210	SD = 3.300
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2. Placebo Group: Mean = 3.6505 SD = 5.090

This information is being used to calculate the sample size for this phase III trial. Since in the previous trial variances (or SDs) was different, sample size for the new trial is calculated using unequal variances. The assumptions are:

- Primary endpoint = net average combined daily rhinoconjunctivitis symptom and medication scores (netCOMBSC) during the entire ragweed pollen season
- Test of significance level (alpha) = 0.05
- Based on 2-sided test
- Power (%) = 90

To calculate the sample size, the following null (H_0) and alternative (H_A) hypotheses were formulated:

$$H_0: \mu_1 = \mu_2 \quad \text{vs.} \quad H_A: \mu_1 \neq \mu_2$$

A sample size of 188 in each group will have 90% power to detect a difference in means of -1.440 (the difference between a SLIT Group netCOMBSC mean, m_1 , of 2.210 and a Placebo Group mean, m_2 , of 3.6505) assuming that the SLIT SD, s_1 , is 3.300 and the Placebo SD, s_2 , is 5.090 using a two group Satterthwaite t-test with a 0.050 two-sided significance level.

The clinically meaningful difference of SLIT over Placebo will be defined as a 20% decrease in netCOMBSC in SLIT from Placebo. As such, the null hypothesis ($H_0: \mu_1 = \mu_2$) will be rejected in favour of the alternate hypothesis ($H_A: \mu_1 \neq \mu_2$) if the p-value of the two-sided t-test is <0.05 and that the point estimate of the treatment means shows a clinically meaningful decrease in netCOMBSC of at least 20% in the SLIT group over Placebo.

Therefore, a total of 376 patients equally divided into two groups would be needed. This number is increased by 10% to account for dropouts, giving a sample size of 418 with 209 patients in each treatment group.

8. ANALYSIS POPULATION

Three populations of Subjects will be defined for purposes of statistical analysis and reporting of study results as follows:

Intent-to-Treat (ITT) Population

The Intent-to-Treat Population will include all randomized subjects who received at least one dose of study medication and have at least one post dose efficacy assessment.

Per Protocol Population

The Per Protocol Population is a subset of the ITT Population. It will include subjects who have met all inclusion and exclusion criteria, have no major protocol violations (as documented in an appendix to the Statistical Analysis Plan prior to unblinding the study), and minimally have completed the following:

1. Subjects must have at least 4 days of valid baseline data in order to be considered evaluable for Per Protocol analyses.
2. Subjects must have at least two valid weeks of data during the entire pollen season in order to be considered evaluable for Per Protocol analyses. A week will be defined as valid if the combined daily rhinoconjunctivitis symptom and medication score could be calculated for 4 of 7 days.

Safety Population

The Safety Population will include all randomized subjects who have received at least one dose of study medication. The safety set will be used in all safety/tolerability analyses. Subjects will be analyzed according to the treatment they received.

9. STUDY ENDPOINTS

Primary Efficacy Variable

The primary efficacy measure will be the net average combined daily rhinoconjunctivitis symptom and medication scores (netCOMBSC) self-recorded daily by subjects in their electronic diaries during the 2011 short ragweed pollen season in the US and Canada.

Secondary Efficacy Variables

- a) The net average combined daily rhinoconjunctivitis symptom and medication score reported during the 3 peak weeks of the short ragweed pollen season (netpCOMBSC)
- b) The net average daily rhinoconjunctivitis symptom score during the entire short ragweed pollen season (netRSS)
- c) The net average daily rhinoconjunctivitis symptom score reported during the 3 peak weeks of the short ragweed pollen (netpRSS)

Exploratory Variables

Exploratory variables will be the pharmacodynamic variables of change in serum concentration of ragweed-specific IgG, IgG₄, and IgE antibodies from screening to final visit.

Safety Variables

Safety will be evaluated by adverse events (AEs) self-reported by subjects, concomitant medication use, vital signs, clinical laboratory evaluations, and changes in physical examinations.

10. STATISTICAL METHODS

If not otherwise specified, statistical significance is defined as $p < 0.05$ and is two-tailed when appropriate.

Data will be summarised with respect to demographic and baseline characteristics, efficacy variables and safety variables.

Summary statistics will include the mean, N, standard deviation, median, minimum, and maximum values for continuous variables, and frequencies and percentages for categorical variables.

10.1 Study Hypotheses

The primary hypothesis of this Phase III trial in adult allergic rhinoconjunctivitis patients is that SLIT with Greer® Standardized Short Ragweed Pollen Allergenic Extract will reduce combined daily rhinoconjunctivitis symptom and medication scores more than placebo during the entire ragweed pollen season.

Secondary hypotheses are that SLIT: reduces combined daily rhinoconjunctivitis symptom and medication scores more than placebo during the three peak weeks of the ragweed pollen season; reduces the rhinoconjunctivitis symptom scores during the entire and/or three peak weeks of the ragweed pollen season; increases ragweed-specific IgG, IgG₄ and IgE levels during the ragweed pollen season; and, is generally safe as compared to placebo.

10.2 Baseline Characteristics

10.2.1 Demographics

Baseline measures and demographic information will be summarized for each treatment group. Descriptive statistics will be reported for each measure, but no formal statistical testing will be performed to compare the treatment groups. The number of observations, mean, standard deviation, median, minimum and maximum will be reported for continuous measures (e.g., age), whereas categorical outcomes (e.g., gender) will be described with frequencies/percents.

Age is calculated in years without rounding as follows:

Year of age = (Date informed consent signed - Date of birth) / 365.25

All data will be listed by treatment and subject.

10.2.2 Medical History

Medical history data recorded at Screening will be listed by treatment and subject.

10.2.3 Skin Prick Test

Skin prick test will be performed at Screening. All data will be listed by treatment and subject.

10.2.4 Pregnancy Test

Urine HCG pregnancy test will be performed at Screening and study completion. All data will be listed by treatment and subject.

10.3 Primary Efficacy Analyses

10.3.1 Primary Efficacy Measure

The primary efficacy measure will be the net average combined daily rhinoconjunctivitis symptom and medication scores (netCOMBSC) self-recorded daily by subjects in their electronic diaries during the 2011 short ragweed pollen season in the US and Canada.

Start of ragweed pollen season is defined as the Monday prior to the official start of ragweed pollen season (see protocol section 7.2.2 for the definition of the official start of ragweed season) for each site. End of ragweed pollen season is defined as the Sunday after the official end of ragweed pollen season for each site (see protocol section 7.2.2 for the definition of the official end of ragweed season).

Directly after preliminary dosing, and continuing through ragweed season, each day subjects will record overnight symptoms in the electronic diary twice: (1) immediately prior to taking their study dose in the morning (AM), and (2) approximately 10-14 hours after dosing to capture symptoms experienced during the day/evening (PM). Each of these two evaluations will be done by subjects reflecting back on the entire period of time since recording their last symptom assessment.

The eight individual allergy symptoms collected in the diaries will be ocular (itchiness, swelling/redness, and watery eyes/tears), nasal (sneezing, itching, runny, and stuffy nose), and ears (itching). Each of the symptoms will be rated by subjects on an ordinal scale:

0 = No signs or symptoms evident

1 = Mild symptoms: Sign/symptom clearly present, but minimal awareness; easily tolerated

2 = Moderate symptoms: Definite awareness of sign/symptom that is bothersome but tolerable.

3 = Severe symptoms: Sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping; need for anti-allergy medication.

Net average combined daily rhinoconjunctivitis symptom and medication scores (netCOMBSC) for the entire pollen season will be computed using netRSS + netARM. NetRSS and netARM will be calculated as follows:

RSS: The average daily symptom scores (AM/PM) for the entire pollen season will be computed first by taking the mean of the daily scores (AM and PM). The resulting means will be summed and will then be divided by the number of days for which RSS were taken in the entire pollen season, for an overall mean daily symptom score. If there is only one recording on any day, that single recording will be used for that day. This average will be the RSS.

RSSBL: The average daily symptom scores (AM/PM) for the week prior to the ragweed pollen season will be computed first by taking the mean of the daily scores (AM and PM). The resulting means will be summed and will then be divided by the number of days for which baseline RSS were taken (to a maximum of seven), for an overall mean daily symptom score at baseline. If there is only a single RSS on a day, then that will be the one used for that day. This average will be the RSSBL.

Net average daily symptom scores for the entire short ragweed pollen season, netRSS, will be computed by subtracting RSSBL from RSS:

$$\text{netRSS} = \text{RSS} - \text{RSSBL}$$

Rescue medication Scoring

Specific relief medications used during the study will be self-recorded once daily by subjects in their electronic diary. The associated relief medication scores assigned to medication use are:

- 1 point for using the once-daily oral antihistamine [Claritin® loratadine; 10 mg tablet, once daily]

- 1 point for using the once-daily ocular antihistamine [Pataday® (olopatadine): 1 gtts each eye, once daily]; or the equivalent twice daily Patanol® (1 gtts each eye, twice daily)]
- 1 point for using a treatment with albuterol [1-2 puffs of albuterol (90 mcg/puff) is one application]. Note: This medication will not be scored if it is used as prophylaxis against exercise-induced asthma

ARM: The average daily allergy relief medication scores during the entire short ragweed pollen season will be computed taking the sum of the total daily allergy relief medication scores divided by the number of days for which data will be available.

ARMBL: The average daily allergy relief medication scores for the week prior to the ragweed pollen season will be computed by summing the total daily allergy relief medication scores divided by the number of days for which data will be available. This average will be the ARMBL.

$$\text{netARM} = \text{ARM} - \text{ARMBL}$$

Using the netRSS and net ARM, the primary endpoint, net average combined daily rhinoconjunctivitis symptom and medication score (netCOMBSC) for the entire pollen season (RSS) will be computed as follows:

$$\text{netCOMBSC} = \text{netRSS} + \text{netARM}$$

10.3.2 Primary Efficacy Analysis Method

Response Variables

The primary efficacy variable will be the net average combined daily rhinoconjunctivitis symptom and medication scores (netCOMBSC) recorded during the entire ragweed pollen season. NetCOMBSC during the entire pollen season will be computed for each subject as defined in Section 10.3.1.

Descriptive Statistics

Descriptive statistics (number, mean, standard deviation, median, minimum and maximum) will be provided for baseline, post treatment, and change from baseline by treatment.

ANCOVA Model

First a test comparing the variances in the two groups will be performed. Next, an Analysis of Covariance (ANCOVA) or, if necessary, a test with unequal variances will be performed to compare the two treatment groups using netCOMBSC as the dependent variable and

baseline (RSSBL+ARMBL) as the covariate. In addition, center will be included in the final model as a covariate.

The LSMeans for each treatment, the LSMeans of the treatment difference together with the corresponding 95%-confidence interval and the p-value will be presented.

Assessment of Interactions

The interactions of treatment-by-baseline and treatment-by-center will be assessed in two separate models using 2 two-way analysis of covariance models with the effects of treatment, center and baseline common to both.

A non-significant interaction effect at the 0.10 level of significance or a significant interaction that is only quantitative and not qualitative in nature (i.e. where the treatment effect differs only in magnitude but not direction across study centers) will support the pooling of subjects across study centers for purposes of the primary analysis pairwise comparisons. Centers with 5 or less subjects randomized will be pooled with the closest geographic site (by distance between the two sites).

Nonparametric Procedures

If the data warrants nonparametric procedures to be employed, the Wilcoxon's 2- sample test for comparison of both treatment groups (SLIT vs. placebo) will be used. The treatment effect will be estimated by the median value of the differences of both treatment groups and listed with related nonparametric 95%-confidence interval according to Lehmann & Hodges.

All statistical tests and confidence intervals to compare the two treatments will be two-sided with $\alpha=0.05$. No adjustments to the type I error rate will be done to account for multiple analysis variables, as there is only one primary analysis.

The ITT Population will be used for the primary analysis, although an analysis will be conducted using the Per Protocol Population. Dose adjustments (up/down) may occur throughout the study based on Subject response and Investigator judgment. Regardless of the changes in dose, analyses will be done only considering the SLIT and placebo groups.

Sensitivity Testing

Several methods of sensitivity testing for missing data will be performed for the primary analysis.

5. The Last Observation Carried Forward (LOCF) method will be applied using the last data available prior to drop out, on post-treatment assessments only.
6. The LOCF method will also be performed on AM and PM symptom scores separately.

7. The rate of missing data in the primary endpoint scores will be examined between treatment groups. Kaplan-Meier survival curves will be provided.

8. Matched Pairs

The combined score will be estimated by the following procedure:

At least 5 subjects from the database randomized to the same treatment group as the subject with missing data that fall into the same category with respect to the following classifications are to be found:

- Age (groups: <40 , ≥ 40)
- Sex (male, female)
- IgE (< 0.35 kU/L; $0.35 - 0.7$ kU/L; > 0.7 kU/L)
- Skin prick test (sum of erythema) (< 90 , ≥ 90).

Data from the PP population will be used in creating the different strata. The mean of combined scores within the group of subjects found to match the subject with missing values will be determined. This mean value will be used as an estimate for the outcome of the primary endpoint for the withdrawn subject during the primary efficacy analysis.

Before database closure, matched pairs will be identified for missing values of the primary efficacy endpoint on blinded data. In case matched pairs cannot be found or there are too few subjects (< 5) that fall into the same category with respect to the above mentioned classifications, the classifications will be re-assessed (i.e. collapsing of age group or another strata). Decisions on the revision will be made prior to unblinding.

10.4 Secondary Efficacy Analyses

10.4.1 Secondary Measures

Secondary measures will include:

- a) The net combined daily rhinoconjunctivitis symptom and medication scores reported during the 3 peak weeks of the short ragweed pollen season (netpCOMBSC).

The three peak (highest) weeks of ragweed pollen counts during the entire ragweed pollen season will be contiguous. Three contiguous peak weeks for the particular study site will be determined by calculating a moving average of the available ragweed pollen counts (grains/m³) for each week (Monday -- Sunday). The following smoothing technique to all observation days except the first and the last day of the season will be applied:

The final daily pollen count of day i will be calculated as moving average of the previous $(i-1)^{\text{th}}$ -day, the i^{th} -day and the following $(i+1)^{\text{th}}$ -day (i.e. $\text{count} = \text{average}(\text{count}(\text{day } i-1), \text{count}(\text{day } i) \text{ and } \text{count}(\text{day } i+1))$).

The first and last day of the season will be set to missing. At least 3 counts per week should be available. A weekly mean will then be tabulated on the moving averages based on a Mon-Sun week. At least 3 counts per week should be available. Then based on these weekly means, three peak weeks are chosen in rank order, as follows:

- a) The “maximal” three weeks of pollen counts over the course of the season (non-contiguous method)
- b) The 2 contiguous weeks (of highest pollen counts) + the week following;
- c) The 2 contiguous weeks (of highest pollen counts) + whichever week, before or after, is higher;

There was a critical mitigating factor this ragweed season, and that is the influence of the two tropical storms that dumped a remarkable 15 to 20 inches of rain in sites that were located in the storm belt (N.C. -- mid-Atlantic -- mid-PA -- parts of N.Y. State -- and into VT and Canada). Both storms (TS Irene; TS Lee) occurred in the peak period of the ragweed season. Due to the above mentioned reasons, the analysis based on the three non-contiguous peak weeks during the entire ragweed pollen season will also be performed. Further, the statistical analyses for this secondary outcome variable will be done in rank order using three methods mentioned above.

The net average daily rhinoconjunctivitis symptom scores and the net average daily allergy relief medication scores reported during the 3 peak weeks of the short ragweed pollen season (netpRSS and netpARM, respectively) will be computed similar to the netRSS and netARM with the only exception being the time span used to calculate the scores.

$$\text{netpRSS} = \text{pRSS} - \text{RSSBL}$$

$$\text{netpARM} = \text{pARM} - \text{ARMBL}$$

$$\text{netpCOMBSC} = \text{netpRSS} + \text{netpARM}$$

- b) The net average daily rhinoconjunctivitis symptom score reported during the entire short ragweed pollen (netRSS) as computed in 10.3.1, above.
- c) The net average daily rhinoconjunctivitis symptom score reported during the 3 peak weeks of the short ragweed pollen season (netpRSS) as computed in 10.3.1, above.

10.4.2 Secondary Efficacy Analysis Method

Secondary efficacy analyses will be performed for the secondary measure of net average combined daily rhinoconjunctivitis symptom and medication score reported during the 3 peak weeks of the short ragweed pollen season (netpCOMBSC). Similar methods of

analyses described in section 10.3.2 will be used for this endpoint, and all secondary endpoints that are continuous in nature.

The net average daily rhinoconjunctivitis symptom score during the entire short ragweed pollen season (netRSS) and also for the 3 peak weeks (netpRSS) will be computed for each subject. These secondary efficacy variables will be computed for each Subject as described in section 10.3.2. High scores will be indicative of poor symptom relief from the study medication. The ITT Population and the PP Population will be used for this analysis.

10.5 Safety Analyses

The safety Population will be used for all safety displays.

No statistical comparisons will be made between the treatments for any safety measure. Dose adjustments (up/down) may occur throughout the study based on Subject response and Investigator judgment. Regardless of the changes in dose, analyses will be done only considering the SLIT and placebo groups.

10.5.1 Safety Measure

Safety will be evaluated by adverse events (AEs) self-reported by subjects, concomitant medication use, vital signs, clinical laboratory evaluations, and changes in physical examinations.

10.5.2 Adverse Events

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) central coding dictionary (most recent version). Each adverse event is to be evaluated for date/time of onset, duration, severity and causal relationship with the study drug or other factors.

The frequency of adverse events (pre-treatment AEs) or treatment emergent adverse events (TEAEs) and serious adverse events (SAEs) will be tabulated for each treatment for four time periods: (1) Pre-Dose: Prior to first dose; (2) Pre-season: From first dose until the start of the ragweed season, (3) Ragweed Season: From the start of the ragweed season until end study; and, (4) Overall: From first dose until end study.

TEAEs will be summarized by System Organ Class and Preferred Term (MedDRA) and by severity. Based on the MedDRA preferred term, each AE will be counted only once for a given subject. If the same AE occurs in a subject on multiple occasions, the highest severity and least complementary relationship will be assumed. If two or more AEs are reported as a single event, the individual terms will be reported as separate AEs.

A subgroup analysis by race (white, non-white) and gender (male, female) will be performed for the SAEs.

In addition, the most common TEAEs (TEAEs with an incidence $\geq 5\%$ in any treatment group) will be summarized by system organ class and preferred term, and will be subjected to a subgroup analysis by race (white, non-white) and gender (male, female).

All AEs will be listed for individual by treatment.

All SAEs will be listed.

10.5.3 Vital Signs

Blood pressure, heart rate, respiratory rate and body temperature are recorded at Screening and study completion.

Descriptive summaries (n, mean, standard deviation, minimum, median and maximum) will be provided for the change from baseline value by treatment. Baseline is defined as the screening values.

All data will be listed by treatment and subject.

10.5.4 Clinical Laboratory Tests

Clinical laboratory tests are collected at Screening and study completion.

Data for individual subjects will be listed by treatment and subject. Any out of range values will be flagged.

All safety laboratory assessments will be summarized by treatment. Descriptive summaries (n, mean, standard deviation, minimum, median and maximum) will be provided for the change from baseline value. Baseline is defined as the lab value at Screening.

10.5.5 Physical Examination

Physical examinations will be performed at Screening and study completion. Physical examinations will be listed by treatment and subject.

10.5.6 Concomitant Medications

All prior and concomitant medications taken since screening until study completion will be listed by treatment and subject. Medications will be coded according to the WHO drug dictionary (most recent version), which includes the WHO Drug Name and the ATC Classification.

10.10 Accounting for Missing, Unused, or Spurious Data

Symptom Scores

Symptom scores will be entered twice daily (AM and PM) by Subjects into an electronic diary. The diary contents will be regularly downloaded to a central database that can be monitored for compliance. It is not anticipated that many symptom scores will be missing with this approach to symptom score collection. Therefore, no missing symptom scores will be imputed from other values. If any scores are missing they will remain missing for all computations.

Investigative sites will be selected that have demonstrated historically measurable, consistent ragweed pollen levels. However, poor weather conditions and other factors can adversely affect seasonal ragweed growth and subsequent airborne pollen concentrations. Investigative sites that lack sufficient ragweed pollen to initiate ragweed allergy symptoms will be excluded from analysis. Pollen counts will be reviewed prior to unblinding the study, and excluded sites (if any) will be documented with rationale in an appendix to the Statistical Analysis Plan that will be added and signed off prior to unblinding.

Relief Medication Use

Relief medications used throughout the study will be entered into the electronic diary. Subjects will be instructed on the use of the diary and how to enter medication use. No imputation of missing data will be done.

Ragweed-Specific IgG, IgG4 and IgE Antibodies

Serum from blood samples collected during screening, treatment visits and study completion will be evaluated for ragweed-specific IgG, IgG₄ and IgE antibody levels.

No imputation of missing data will be done for either visit. Extreme outliers may be excluded from analysis, if justified. Values below the quantification limit of the assay will be assigned a small, no zero, value for purposes of analysis.

Clinical Laboratory Assays

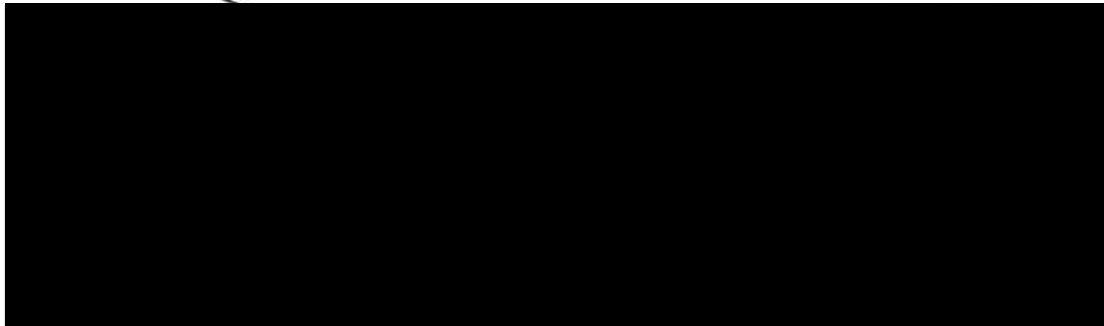
Serum from blood samples collected during screening and study completion (Visit 7) will be assayed for normal/abnormal blood chemistry values. No imputation of missing data will be done for either visit. Extreme outliers may be excluded from analysis, if justified. Values below the quantification limit of the assay will be assigned a small, no zero, value for purposes of analysis.

Adverse Events

Missing or partial start/stop dates for AEs will attempt to be resolved through query of the investigative sites. When that information is unavailable, the closest logical date in which

the AE was present will be used to estimate duration of the event. AEs that continue and change severity will use the most severe grading for reporting purposes.

11. TABLE, LISTING AND FIGURE SHELLS



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APPENDIX A: SCHEDULE OF EVENTS

Procedures

	Screening	Visit 1 ¹				Visit 2 ²	Visit 3 ²	Visit 4 ²	Visit 5 ²	Visit 6 ²	Visit 7 ³
		Day 0									
		0	15 min	30 min	Dose Selection	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Informed Consent/Medical History	X										
Physical Exam	X										X
Spirometry ⁴	X	X ⁵	X ⁵	X ⁵							
Skin Prick Test	X										
Urine HGC Pregnancy Test ⁶	X										X
Blood Draw & Urine collection	X										X
Randomization		X									
Determination of MTD – Dose Escalation		X	X	X							
Assign treatment dose					X						
Report in Electronic Diaries		X	X	X	X	X	X	X	X	X	X
Study supplies and training					X						
Review Electronic Diaries		X	X	X	X	X	X	X	X	X	X
Determination of Dose Adjustment						X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	X	X

¹ Within 8 to 16 weeks of ragweed pollen season start.

² Visits will be scheduled every 30 days ($\pm$ 7 days) apart and conducted no later than 12:30 PM

³ The final visit will be conducted after the completion of the ragweed pollen season defined in Section 7.2.2.

⁴ Asthmatic subjects only.

⁵ Peak flow is to be measured in asthmatics, after each dose given during preliminary dosing. Allow subjects to record their symptoms before measuring lung function via the peak flow meter.

⁶ Females only.

Attachment 1: Medications to be withheld prior to skin testing

Prescription Anti-histamines to be withheld 72 hours prior to study admission	Atrohist, Bromfed, Codimal, Comhist, Deconamine, Extendryl, Kronofed, Marax, Optimine, PBZ, Phenergan, Polyhistine, Proaramine, Rondec, Rynatan, Trinalin, Vistaril, Periactin
Prescription Anti-histamines to be withheld 7 days prior to study admission	Claritin, Clarinex, Allegra, Zyrtec, Xyzal, Astelin, Patanase, Atarax, and all respective combinations e.g., Claritin-D.
Over the counter medications containing Chlorpheniramine to be withheld for 72 hours prior to study admission	Aller-Chlor, C.P.M., Chlor-Amine, Chlor-Mal, Chlor-Penit, Chlor-Trimeton, Teldrin, Chlorphen, Effidac-24, Ridraman
Over the counter medications containing Clemastine to be withheld for 72 hours prior to study admission	Allerhist-1, contact 12 hour Allergy, Tavist-1
Over the counter medications containing Diphenhydramine to be withheld for 72 hours prior to study admission	Actifed, Aler-dryl, Banophren, Benadryl, Calm-Aid, Compoz Nighttime, Sleep-Aid, Diphedryl, Diphen, Genahist, Hydramine, Nu-med, Nytol, Caplet, Scot-tussin Allergy relief formula, Sominex, Twlite, Tylenol PM, Unisom Sleep Gels Drixoral

This table contains examples of drugs and there with holding times prior to skin testing, it is not intended to be a complete list. If you have any questions about drugs and with holding times please contact the sponsor.

Attachment 2: Global Initiative for Asthma (GINA)

Class	Designation	Symptoms	Nocturnal Symptoms	Lung Function
I	Intermittent	Less than once a week Brief exacerbations	≤ twice a month	FEV ₁ or PEF ≥ 80% predicted; PEF or FEV ₁ variability <20%
II	Mild persistent	>once a week but <once a day; exacerbations may affect activity and sleep	> twice a month	FEV ₁ or PEF ≥ 80% predicted; PEF or FEV ₁ variability ≤ 20%–30%
III	Moderate persistent	Daily exacerbations may affect activity and sleep; daily use of inhaled short-acting β_2 -agonist	> once a week	FEV ₁ 60% - 80% predicted; PEF or FEV ₁ variability > 30%
IV	Severe persistent	Daily; frequent exacerbations; limitation of physical activities	Frequent	FEV ₁ or PEF ≤ 60% predicted; PEF or FEV ₁ variability >30%

*The worst feature determines the severity classification

Adapted from GINA Classification: Global Initiative for Asthma. Global strategy for asthma management and prevention. Updated 2008. Accessed at <http://www.ginasthma.org>.