C O N F I D E N T I A L

Immune Tolerance Network

Protocol ITN019AD

Phase II, Double-Blinded, Placebo-Controlled, Efficacy and Safety Evaluation of Allergen Immunotherapy Co-Administered with Omalizumab, an Anti-IgE Monoclonal Antibody

Allergen Immunotherapy Co-Administered with Omalizumab

BB-IND # 9996

Version 8.0 (September 10, 2004)

Confidentiality Statement

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Protocol Approval

Protocol #: ITN019AD	Protocol Version and Date: Version 8.0, September 10, 2004
IND#: BB-IND 9996	Primary Investigator: Thomas B. Casale, MD
Short Title of Protocol: Allergen immunotherapy Co-administered with Omalizumab	

I have read and approve this protocol and agree to conduct it using Good Clinical Practices as delineated in "ICH Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996.

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Principal Investigator Date

Synopsis

Title:	Efficacy and Safety Evaluation of Allergen Immunotherapy Co-Administered with Omalizumab, an anti-IgE Monoclonal Antibody
Short Title:	Allergen Immunotherapy Co-Administered with Omalizumab
Clinical Phase:	II
Sponsors:	NIAID and the Immune Tolerance Network
Principal Investigator:	Thomas B. Casale, M.D.
Participating Site(s):	Creighton University University of Wisconsin, Madison University of Iowa
Accrual Objective:	168 participants (42 participants per arm)
Accrual Period:	Approximately three weeks
Study Design:	This is a double-blinded, parallel group, multi-center, placebo-controlled study. This Phase II study will examine whether pre-treatment of participants with omalizumab followed by rush immunotherapy (RIT), followed by dual therapy with omalizumab plus immunotherapy (IT) will be safer and more effective in preventing symptoms in ragweed-induced seasonal allergic rhinitis (SAR) versus omalizumab alone, IT alone or placebo. Omalizumab (or placebo) will be given every 2 or 4 weeks prior to RIT or placebo RIT. RIT must be completed at least 3 weeks prior to the start of ragweed season. After RIT, participants will receive weekly maintenance ragweed IT or placebo IT, and omalizumab or placebo, every 2 or 4 weeks for 12 weeks. These 12 weeks will begin prior to the ragweed season, continue through the ragweed season, and for some participants, may extend beyond the ragweed season. A follow-up period (including an extended follow-up phase during the 2004 ragweed season) will examine whether persistent immunologic and clinical tolerance has been achieved. Participants will be randomized (1:1:1:1) to 4 treatment groups as follows: 1. Omalizumab pre-treatment, ragweed RIT, omalizumab + ragweed IT 2. Omalizumab pre-treatment, placebo RIT, omalizumab + placebo IT 3. Placebo omalizumab pre-treatment, ragweed RIT, placebo omalizumab + ragweed IT 4. Placebo omalizumab pre-treatment, placebo RIT, placebo omalizumab + placebo IT
Study Duration:	Approximately 55 weeks, that includes screening, pre-treatment, treatment, and primary follow-up (visits 1 through 20 from April 2003 through April/ May 2004). **Extended follow-up phase,** 28 weeks: Those participants who have received all study treatments will be asked to sign an addendum to the informed consent at visit 20 (or the soonest convenient time thereafter) to participate in the extended follow-up phase. Visits 21 through 28 will occur starting approximately one month before the ragweed season in 2004 (~early July 2004) and end at least 2 weeks into the post-ragweed season.
Primary Study Objective:	To examine whether omalizumab given prior to RIT followed by 12 weeks of dual omalizumab and IT is more effective than RIT followed by IT alone in preventing the symptoms of ragweed-induced SAR.

Secondary Study Objectives: To examine whether omalizumab given prior to RIT followed by 12 weeks of dual omalizumab and IT is safe and more effective than omalizumab alone or placebo in preventing the symptoms of ragweed-induced SAR; to assess the immunologic mechanisms associated with the therapies; and to assess whether clinical tolerance has been achieved after discontinuation of the therapies.

Primary Endpoint: The primary endpoint will be the average daily allergy severity score, which will be calculated from participants' 5 symptom scores (sneezing; rhinorrhea/runny nose; itchy nose, throat, and palate; itchy, watery eyes; and nasal congestion/stuffiness) during the 2003 ragweed pollen season. Symptom scores are recorded twice daily (AM and PM)**.** The ragweed pollen season begins when the ragweed pollen counts rise to 10 ragweed pollen grains/m^3/24 hours or above on two consecutive recorded days, and the ragweed pollen season ends when the ragweed pollen counts fall below 10 ragweed pollen grains/m^3/24 hours on two consecutive recorded days. The ragweed pollen season is from approximately August 15, 2003 to October 1, 2003, but varies among the sites. The sum of the individual symptom scores will be averaged over AM and PM to give a daily score. Each daily score will then be averaged to obtain one measure of the average daily allergy severity score for each participant.

Secondary Endpoints: The secondary efficacy variables are:

1. The incidence and severity of adverse events;
2. Number of days with rescue medication use during the 2003 ragweed season;
3. Number of rescue medication capsules (fexofenadine HCl 60 mg) used during the 2003 ragweed season;
4. Rhinoconjunctivitis QOL questionnaire (RQLQ) scores during the 2003 ragweed season;
5. Daily AM allergy symptom scores during the 2003 ragweed season;
6. Daily PM allergy symptom scores during the 2003 ragweed season; and
7. Individual allergy symptom scores during the 2003 ragweed season.

The effects of Study Treatment on inducing an immune tolerant state will be explored through *in vivo* and *in vitro* immunologic tests. These exploratory endpoints will examine the effects of Study Treatment on shifting the ragweed-induced immune response toward a Th1 response and inhibition of early and late phase response to ragweed-induced allergy skin tests and nasal allergen challenges. Please refer to Section 9 for a complete discussion of tolerance assays studies.

Tertiary Endpoints

1. Average daily allergy severity score (as described above) during the 2004 season.
2. Change in average daily allergy severity scores between the 2003 and 2004 ragweed seasons.
3. Number of days with rescue medication use during the 2004 ragweed season;
4. Number of rescue medication capsules (fexofenadine HCl 60 mg) used during the 2004 ragweed season;
5. Rhinoconjunctivitis QOL questionnaire (RQLQ) scores during the 2004 ragweed season;
6. Daily AM allergy symptom scores during the 2004 ragweed season;
7. Daily PM allergy symptom scores during the 2004 ragweed season; and
8. Individual allergy symptom scores during the 2004 ragweed season.
9. Assessment of ragweed-specific IgG and IgE levels throughout the study.
10. Assessment of other immunologic parameters as outlined in section 9.

Inclusion Criteria:

1. Able to comprehend and grant a witnessed, written informed consent prior to any study procedures.
2. Male or female 18 to 50 years of age.
3. Female participants of child bearing age must have a negative urine pregnancy test at Visit -01 and a negative urine pregnancy test at subsequent visits. In addition, female participants must be using a medically acceptable form of birth control.
4. History of seasonal allergic rhinitis for at least 2 years with symptoms during the ragweed pollen season requiring pharmacotherapy.
5. A positive skin test by prick method to ragweed pollen at Visit -01. A positive skin prick test will be defined as a ragweed pollen-induced wheal >3 mm larger in diameter than diluent control (measurements will be made 15-20 minutes after application).
6. Must be capable of faithfully completing the diary and of attending regularly scheduled study visits.
7. Must intend to remain in the ragweed pollen area during the entire ragweed season.
8. Willing to avoid prohibited medications for the periods indicated in the protocol.
9. Participants must meet pretrial eligibility requirements for trial enrollment (acceptable medical history, physical examination results, normal electrocardiogram and acceptable laboratory test results).
10. Participants must have a baseline serum IgE level > 10 and < 700 IU/mL.

Exclusion Criteria:

1. Participants < 30 kg or > 120 kg.
2. Participants who are pregnant or lactating.
3. Participants with a history of severe anaphylactoid (non-IgE mediated) or anaphylactic reaction(s).
4. Participants with a history of immunotherapy within the past 10 years, if received one full year of immunotherapy, or within the past 5 years if received less than one year of immunotherapy.
5. Participants with known hypersensitivity to trial rescue medication (fexofenadine HCl).
6. Participants taking beta-adrenergic antagonists in any form.
7. Participants taking allergic ophthalmologic medication
8. Participants with clinically significant perennial rhinitis that would interfere in assessment of ragweed-induced seasonal allergic rhinitis symptoms.
9. Presence of a severely deviated nasal septum, septal perforation, structural nasal defect or large nasal polyps causing obstruction.
10. History of an upper respiratory or sinus infection requiring treatment with an antibiotic within 2 weeks prior to Visit -01.
11. Documented evidence of acute or significant chronic sinusitis, as determined by the Investigator.
12. Asthma (either history of, abnormal spirometry, [FEV1 <80% predicted] or use of asthma medications).
13. Chronic or intermittent use of inhaled, oral, intra-muscular, or intra-venous corticosteroids; or chronic or intermittent use of topical corticosteroids within 4 weeks of Visit -01.
14. Chronic use of medications (e.g., tricyclic antidepressants) that would affect assessment of the effectiveness of the study medication.
15. Rhinitis medicamentosa.
16. History or presence of significant renal, hepatic, neurologic, cardiovascular, hematologic, metabolic, cerebrovascular, respiratory, gastrointestinal or other significant medical condition including, autoimmune or collagen vascular disorders, aside from organ-specific autoimmune disease limited to the thyroid that in the Investigator's opinion could interfere with the study or require medical treatment that would interfere with the study.
17. History of cancer other than basal cell carcinoma of the skin.
18. History within the past year of excessive alcohol intake or drug addiction.
19. Current smokers, greater than 10 pack year history, or participants who quit smoking less than one year prior to Screening.
20. Use of any prohibited concomitant medications during the washout period (i.e., before screening) and throughout the study period.
21. Participants currently undergoing immunotherapy.
22. Participants with clinically significant abnormality on 12-lead ECG on screening visit.
23. Treatment with an experimental, non-approved drug, or investigational drug within the past 30 days.
24. Participants with a history of noncompliance to medical regimens and participants who are considered potentially unreliable.
25. Previous treatment with a monoclonal antibody for any reason including anti-IgE in any form (e.g., omalizumab).
26. Participants with known hypersensitivity to trial drug ingredients (i.e., sucrose, histidine, polysorbate 20) or related drugs (i.e., monoclonal antibody; polyclonal gamma-globulin).

Treatment Description:

Participants will receive a combination of omalizumab and immunotherapy or matching placebo. Omalizumab reduces the amount of free IgE (circulating or unbound) that is available to bind to FCεR1 receptors on mast cells, basophils and other cells, leading to reduction in IgE-mediated allergic symptoms. Omalizumab does not bind to IgE already bound to effector cells, and therefore does not cause anaphylaxis. Participants will receive subcutaneous injections of omalizumab at a minimum dose of 0.016 mg/kg/IgE (IU/mL) for the 4-week dosing interval or a minimum dose of 0.008 mg/kg/IgE (IU/mL) for the 2-week dosing interval, depending on the body weight and the baseline IgE level (IU/mL). In addition, participants will receive immunotherapy consisting of a series of injections containing ragweed extract. A rush IT protocol will be utilized whereby the series of dilutions will be achieved in one day according to the table in Appendix 3. This will be followed by 12 weekly IT injections in combination with every 2 or 4-week omalizumab injections.

Summary of Study Procedures:

The study is divided into 6 periods:

1. Screening and baseline (3 weeks);
2. Omalizumab pre-treatment (9 weeks);
3. Rush immunotherapy (1 day);
4. Omalizumab/placebo and immunotherapy/placebo (12 weeks); and
5. Primary Follow-up (31 weeks).
6. Extended follow-up phase for the 2004 ragweed season (28 weeks)

During screening, participant eligibility will be assessed. All participants must sign an informed consent prior to any protocol screening studies. During the pre-treatment period, participants will receive SC injections of omalizumab or matching placebo every 2 or 4 weeks. During RIT, participants will receive escalating doses of ragweed extract according to the table in Appendix 3 during which the vital signs will be assessed every 30 minutes. All participants will have multiple safety evaluations prior to each injection, recording any and all adverse events. Participants randomized to receive placebo RIT will receive up to 0.125 mg/mL histamine. Participants will be pre-treated by 180 mg of fexofenadine 24 (at home) and 1 hour prior to RIT administration. RIT must be completed at least 3 weeks prior to the start of ragweed season. Participants will receive weekly IT and omalizumab every 2 or 4 weeks for 12 weeks post-RIT.

Primary follow-up phase: Participants will be followed for 43 weeks post-RIT. During this period, safety and efficacy (including immune tolerance) will be assessed.

Extended follow-up phase: Participants who have received all study treatments will be followed up during the 2004 ragweed season (~July 2004 through October 2004). These participants will be asked to sign an addendum to the informed consent at visit 20 (or the soonest convenient time thereafter). During this period, participants will be issued diary cards to record symptom scores and undergo other procedures per the schedule of assessment in Appendix 4, visits 21 through 28. Participants must follow all the restrictions and stipulations per protocol.

Statistical Consideration:

The sample size (168 participants, 42 per treatment arm) was calculated based on previous experience with the following assumptions: a mean difference of 0.40 between the study groups, $p < 0.05$, power of 80%, and a dropout rate of 30%. Up to 300 potential participants will be screened in order to randomize 168 eligible participants.

The data analyses will be based primarily on the intent-to-treat population, with the exception that the tertiary analyses will be based on the per-protocol population by study design. The primary analysis will compare the combination treatment of omalizumab + IT versus IT alone. Secondary analyses will compare each treatment group versus the omalizumab + ragweed IT treatment group. Tertiary analyses will compare each treatment group versus the placebo treatment group.

Table of Contents

Glossary of Abbreviations

AE	Adverse Event
ANOVA	Analysis of Variance
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CTC	Common Toxicity Criteria
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ELISPOT	Enzyme-linked Immunospot
FEV	Forced expiratory volume
GCP	Good Clinical Practice
HAHA	Human anti-human antibodies
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
IND	Investigational New Drug
IRB	Institutional review board
IT	Immunotherapy
ITT	Intent-to-treat
ITN	Immune Tolerance Network
IV	Intravenous
mAb	Monoclonal antibody
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NO	Nitric Oxide
PCR	Polymerase chain reaction
PBMC	Peripheral Blood Mononuclear Cells
PP	Per-protocol
QOL	Quality of life
RES	Reticuloendothelial system
RIT	Rush immunotherapy
RQLQ	Rhinoconjunctivitis QOL questionnaire
SAE	Serious adverse event

SAEC	Safety Serious Adverse Event Coordinator
SAR	Seasonal allergic rhinitis
SC	Subcutaneous
SMT	Study Management Team
TAG	Tolerance Assay Group

1 Background Information and Scientific Rationale

1.1 Background

Allergic rhinitis affects 20 to 40 million Americans annually, including 10 to 30% of adults and up to 40% of children.[1] Symptoms can range from mild to seriously debilitating and can affect quality of life (QOL) resulting in loss of work or school days.[2] Effective management of seasonal allergic rhinitis (SAR) may be an important component of the treatment of co-existing or complicating respiratory conditions, i.e., asthma, sinusitis, and otitis media.[3]

Current therapies for SAR include allergen avoidance, pharmacological interventions such as antihistamines, sympathomimetics, topical and systemic corticosteroids, and chromones, and immunotherapy.[4] Although pharmacologic agents are effective for some participants, their role is limited by their inability to completely relieve symptoms, and in some cases, the induction of deleterious side effects. Immunotherapy (IT) regimens can be highly effective in controlling symptoms of SAR and can offer advantages over pharmacotherapy for those participants who have symptoms that are refractory to medications or those who cannot tolerate the side effects.[1;5] However, IT is associated with the risk of allergic reactions to the extract injections and its effectiveness for allergic respiratory disorders is not always evident, especially in asthma.[6;7] Antibodies against IgE (Immunoglobulin E) have been shown to partially, but not completely ameliorate the symptoms of allergic respiratory disorders.[8-15] Omalizumab, an anti IgE monoclonal antibody, has been shown to be an effective treatment of SAR due to various pollens, including ragweed.[9;14;15] A significant association has been established between omalizumab dose, IgE reduction, and nasal symptom relief;[15] however, upon discontinuation of anti-IgE, serum IgE levels return to pre-treatment levels. Therefore, anti-IgE therapy alone is unlikely to promote long lasting effects. Although, there are some indications that IT alone might induce an immune tolerant state, perhaps through a shift in the Th2 to Th1 immune response profile,[16-20] there are no studies examining long lasting immune tolerance as a result of anti-IgE therapy or combination of IT and anti-IgE. Thus, there is a need for safer and more effective therapies capable of inducing an immune tolerant state. The combination of anti-IgE and allergen IT holds promise as one such therapy.

This protocol hypothesizes that pre-treatment of ragweed allergic rhinitis participants with anti-IgE (omalizumab) will condition the recipient so that subsequent administration of ragweed allergen IT is safer, clinically more effective, and immunologically more efficient at inducing a long lasting immune response to ragweed, hence inducing an immune tolerance state. These effects might in part result from the ability of omalizumab to decrease serum IgE levels and expression of FcεRI and CD23 on critical immune effector cells.
Pre-treatment of allergic participants by omalizumab preceding allergen IT should enhance allergen IT safety by a substantial decrease in free IgE level associated with most severe allergic reactions. Concomitant use of omalizumab with allergen IT should reduce the adverse events associated with IT, improve its effectiveness, and favor development of an

immune tolerant state. Combination therapy should be clinically more effective than either therapy alone. There may be a synergistic effect since both modalities have been shown to be independently effective at reducing symptoms of allergic respiratory disorders via different immunologic mechanisms.

1.2 Description of Investigational Product

In this study, participants will be pretreated with omalizumab or placebo and receive a combination of omalizumab, rush immunotherapy and maintenance immunotherapy.

1.2.1 Omalizumab

Xolair™ (omalizumab) is a recombinant humanized non-anaphylactogenic monoclonal antibody (E25) to IgE, and it has been shown to block the biologic effects of IgE.[8-13] Omalizumab contains 5% murine and 95% human sequence. The human sequence is an IgG1 kappa framework. Omalizumab displays a high affinity for a specific epitope within the C3 domain of IgE, the site of binding of the FcεR1 receptor.[10;21;22] Omalizumab reduces the amount of free IgE (circulating or unbound) that is available to bind to FCεR1 receptors on mast cells, basophils and other cells,[23-25] leading to reduction in IgE-mediated allergic symptoms.[9] When omalizumab binds to free IgE in the systemic circulation and in tissues, it forms small complexes, usually trimers of 2 omalizumab molecules and 1 IgE that are biologically inert and cleared via the RES.[13] Omalizumab does not bind to IgE already bound to effector cells, and therefore does not cause anaphylaxis.

Omalizumab is administered in two separate phases. In the pre-treatment period omalizumab will be administered to condition the participants. Omalizumab will be also administered after RIT and during the maintenance immunotherapy phase.

1.2.2 Immunotherapy

A rush immunotherapy (RIT) protocol will be followed, starting with a 1:1000 dilution of the maintenance vial of ragweed extract. RIT will consist of a series of injections containing ragweed extract. The series of injections will have progressively greater amounts of ragweed extract: starting from the 1:1000 dilution of the maintenance vial and progressing to the 0.3 mL of 1:10 dilution of the maintenance vial or the maximally tolerated amount. With RIT the series of dilutions will be achieved according to the table in Appendix 3. Maintenance immunotherapy that follows RIT will consist of 12 weekly injections of ragweed extract up to the maximally tolerated amount or 0.3 mL of the maintenance vial (1:1 maintenance dilution)

1.3 Summary of Pre-Clinical and Clinical Experience

1.3.1 Early Pre-Clinical Data

Mice sensitized to dust mites and treated with anti-IgE showed significantly reduced lung eosinophilia following allergen challenge and a diminished production of IL-5 by airway Th2 cells. These data suggest that anti-IgE had greater immunologic effects than just the reduction of IgE.[26] In pre-clinical studies omalizumab markedly reduced the density of high

affinity IgE receptors on human basophils (approximately 97%) concomitant with a reduction in serum free-IgE levels. Basophils from these participants showed a 90% reduction in histamine release after challenge with antigen, providing evidence that IgE receptor density is correlated with *in vitro* allergic responses.[27] Omalizumab suppressed allergen-induced wheal and flare skin test responses. Omalizumab also inhibited both early and late-phase asthmatic responses in participants with asthma and reduced eosinophils in induced sputum both pre-and post-allergen challenge, indicating an anti-inflammatory effect.[28;29]

1.3.2 Clinical Experience with Omalizumab for Seasonal Allergic Rhinitis

There have been 4 seasonal allergic rhinitis (SAR) studies examining the therapeutic effectiveness and safety of omalizumab. The first was a Phase II, double-blind, placebo-controlled study in 240 ragweed allergen participants of 18-66 years old.[30] Participants received multiple doses of omalizumab either intravenously (IV) or subcutaneously (SC) with a maximum dose of 0.5 mg/kg IV. The reported adverse events (AE) were generally mild to moderate in severity and there were no significant differences in AE among the omalizumab and placebo groups. No participants were found to have human anti-human antibodies (HAHA) to omalizumab. Omalizumab induced a rapid, dose-dependent reduction in serum free IgE that was related to the baseline IgE levels. Consistent suppression of serum free IgE to the lowest levels of detection required an initial omalizumab: total IgE ratio of approximately 10 or 15:1. This implied that omalizumab dosing must be calculated based on individual baseline IgE levels, not just body weight. Omalizumab had a terminal half-life of approximately 22 days. Only 11 participants at the highest dose experienced a suppression of IgE to undetectable levels. Thus, most participants did not have a significant reduction in symptoms.[30]

Based on these findings, a 12-week, multi-center, randomized, double blind, dose-ranging, placebo-controlled study was carried out in the United States.[15] Five hundred thirty-six (536) ragweed allergen participants were randomized to receive omalizumab 50 mg, 150 mg, 300 mg or placebo SC starting just prior to ragweed season and continuing every 3 or 4 weeks throughout the pollen season. Dosing was stratified based on IgE levels. Omalizumab produced dose-related reductions in serum free IgE levels and improvements in symptoms, rescue antihistamine use, and quality of life (QOL). The 300 mg group showed significant improvements for severity and duration of nasal and ocular symptoms and a reduction in the median proportion of minimal symptom days versus placebo (41 versus 18%, p=0.001). A significant association was observed between IgE reduction and lower nasal symptoms and rescue antihistamine use. The frequency of AEs was similar in the omalizumab and placebo groups. As an extension to this, study participants who had received omalizumab were retreated the subsequent ragweed season. Omalizumab 300 mg was administered SC every 3 or 4 weeks for 12 weeks. The overall incidence and pattern of AE were similar to those reported in the primary study. There were no severe serious AE related to omalizumab and no HAHA were detected. Thus, re-treatment during the second pollen season with omalizumab was well tolerated and not associated with significant immunological problems.[31]

Based on the results of the ragweed studies, 300 mg omalizumab was studied for the treatment of birch pollen-induced SAR in Scandinavia.[14] Two hundred fifty (250) participants were randomly assigned to receive multiple doses of omalizumab or placebo. The frequency of drug or placebo administration was based on baseline total IgE levels. Omalizumab was again well tolerated with no significant differences in either overall AE or trial drug-related AE compared with placebo. No HAHA were detected. Omalizumab significantly lowered average daily nasal and ocular symptoms, number of rescue antihistamines used per day, and proportion of days with any SAR medication use and improved QOL. After discontinuation of omalizumab, IgE levels return to normal over the subsequent 2 to 3 months.[14]

1.3.3 Clinical Experience with Omalizumab Plus Immunotherapy

The safety, tolerability and efficacy of adding omalizumab to IT versus IT alone for SAR due to birch and grass pollen has been previously studied.[32] In this study participants received IT up to a maintenance dosage over a 12-week period. Participants were then randomized to receive either omalizumab or placebo as an add-on therapy for 24 additional weeks. Inclusion criteria included 6 to 17 years of age (mean = 12), 2-year history of moderate to severe SAR due to birch and grass pollen, FEV1 values > 70% and serum IgE levels > 30 and < 1300 IU/mL. This study included 221 participants with 54 to 59 participants in each of 4 groups: birch IT plus omalizumab; birch IT plus placebo; grass IT plus omalizumab; and grass IT plus placebo. The combination of IT plus omalizumab versus IT alone produced a reduction in: (1) SAR symptoms of 35% for birch and 45% for grass; (2) rescue medication scores of 78% for birch and 81% for grass IT; (3) SAR symptom load of 48% for both grass and birch groups; and a safety profile at least as good as IT alone. Thus, these data suggest that the combination of IT and omalizumab is more effective than IT alone.

1.3.4 Clinical Experience with Omalizumab for Asthma

Phase II studies indicated that omalizumab was safe and effective for the treatment of allergic asthma. Omalizumab was found to reduce asthma symptoms and prevent exacerbations in adults and adolescents with moderate to severe asthma, even while corticosteroid and beta agonist use was reduced.[33]

Three pivotal Phase II studies were carried out for asthma in participants 6-75 years old.[34-36] These were randomized, double blind, multi-center, parallel-group studies comparing omalizumab versus placebo administered SC. The study populations included symptomatic adults and adolescents treated with inhaled corticosteroids for 2 studies and asymptomatic children treated with inhaled corticosteroids for the third study. Omalizumab dosing was tailored to baseline body weight and serum IgE levels (approximately 0.016 mg/kg/IgE [IU/mL]). This dose has been demonstrated to reduce serum free IgE levels to lower levels of detection. These trials consisted of 4 phases, a run-in phase of 4 to 6 weeks prior to randomization, a steroid stable phase of 16 weeks during which participants received omalizumab or placebo added on to their standard therapy; a steroid withdrawal phase of 12 weeks; and a 5-month extension safety period. Approximately 1300 participants enrolled

and 767 received omalizumab. Omalizumab reduced the frequency of exacerbations during the add-on and corticosteroid withdrawal phases and omalizumab treated participants demonstrated a greater inhaled corticosteroid reduction. Omalizumab improved standard symptom scores, QOL and pulmonary functions and reduced the frequency of urgent care visits. No significant AE were reported. AE profiles were similar in omalizumab and placebo-treated groups. No reported anaphylactic reactions or serum sickness were observed.

Finally, in a fourth study omalizumab was found to result in a clinically significant reduction of high dose corticosteroids in previously steroid-dependent severe asthmatics without concomitant worsening of asthma exacerbation rate, symptom control and rescue inhaler use, and improved morning peak expiratory flow and QOL. Omalizumab was shown to have an excellent safety and tolerability profile.[37]

1.4 Summary of Known and Potential Risks and Benefits to Human Participants

1.4.1 Immunotherapy

Immunotherapy with either conventional or RIT protocol is used in clinical practice and many research protocols. RIT is an acceptable method for achieving a maintenance dose of allergen immunotherapy in a relatively short period of time. Although both conventional and RIT are associated with risks for the development of non-fatal and fatal systemic reactions, the risk of generalized allergic reactions is greater with RIT than conventional IT. In the WHO position paper on Allergen Immunotherapy: Therapeutic Vaccines for Allergic Diseases" published in 1998, several risk factors were identified for both non-fatal and fatal reactions to immunotherapy.[18] Significant risk factors include:

1. Errors in dosage;
2. Presence of symptomatic asthma;
3. High degree of hypersensitivity by skin test or specific IgE measurements;
4. Use of beta-blockers;
5. Injections from new vials; and
6. Injections made during periods of exacerbations of symptom.

In order to reduce the risks for systemic reactions to rush immunotherapy, we will exclude subjects who have the following: the presence of asthma either symptomatic or asymptomatic; the use of beta-blockers; and injections during periods of exacerbation of symptom. The latter factor is true because patients will receive rush immunotherapy approximately one month prior to the onset of ragweed season. In addition, all immunotherapy doses will be administered by trained personnel and formulated by an allergy extract technician at each site who has experience in making immunotherapy vaccines.

In order to further reduce risks, we will premedicate participants with antihistamines. As stated in the World Health Organization paper, antihistamine have been shown convincingly to reduce the prevalence of systemic side effects.[18]

Despite these precautions, we recognize there is still a risk for the development of acute systemic reactions. The exact risk is not clear, but it does appear higher with rush immunotherapy protocols versus conventional protocols. In an editorial in the Annals of Allergy published in 1994, [38] the incidence of systemic reactions per patient course of rush immunotherapy for asthmatic and/or rhinitic patients who received aqueous extracts range from 15.4% to 66.7%. [38-41] The largest series of 290 patients receiving rush immunotherapy over 3 days resulted in 36.2% developing systemic reactions.[39] In that study published by Hejjaoui, et al, the incidence for systemic reactions per injections was 3.8% with onset of allergic manifestations in the first 45 minutes. In comparison, when pre-treatment was administered, rush immunotherapy has been associated with from 7.3% to 16.2% of patients experiencing systemic reactions and a per injection reaction rate of 0.8% to 3.1%. [39;41;42] Thus, pre-treatment with antihistamines can markedly reduce the incidence of acute allergic reactions to the rush immunotherapy. Conventional allergen immunotherapy has been associated with anaphylactic reactions in 0.8% to 46.7% of patients with a per injection rate of 0.05% to 3.2%. [38;43] This rate approximates that which was found in the rush immunotherapy studies where patients were pretreated. None of the quoted studies controlled for factors such as asthma. Indeed, in all of the studies quoted, patients were more likely to have acute anaphylactic events if they had asthma.

In the participants who receive omalizumab prior to rush immunotherapy, they should have a reduced risk of allergic reactions since by decreasing IgE and high affinity IgE receptors the ability to crosslink IgE receptors in response to ragweed administration will be markedly diminished. For safety precautions taken during this trial to minimize and treat systemic reactions, please refer to Appendix 3.

In summary, the risks for rush immunotherapy after pre-treatment with antihistamine approximates that with conventional immunotherapy. By avoiding high risk patients, having appropriate therapies available for the treatment of allergic reactions, and intensive monitoring of patients at every stage of the immunotherapy protocol, it is anticipated that the risk for study participants will be substantially lowered to acceptable levels mimicking those traditionally observed in private practice settings where allergen immunotherapy is given.

1.4.2 Omalizumab

1.4.2.1 Reported Adverse Events in Humans

The effects of omalizumab, including the inactivation of IgE, are not fully known. Reported adverse events in several Phase I/II/III studies have been comparable between the omalizumab and placebo group. The most common side effects reported in previous studies were viral infections, upper respiratory infection, and headache, regardless of their relationship to omalizumab. These side effects may or may not have been related to omalizumab. In over 2500 participants already dosed with omalizumab, the following events were reported by investigators: headache, back pain, nausea, diarrhea, lightheadedness or dizziness, anxiety, fever, vomiting, generalized pain, bruising (at injection site), painful menstruation, and urticaria (hives) with the first dose of omalizumab. These events were

reported as severe in some individual participants, and all of these events were observed in some participants. However, except for the occurrence of urticaria (hives), there were no obvious dose-related patterns of events, and similar events were also reported in participants receiving only placebo. One participant, treated with omalizumab, experienced dry mouth and worsening of dry eyes (a previously existing condition) similar to Sjogren's-like syndrome. The symptoms may or may not have been due to omalizumab.

The risk for serious adverse event is minimal with omalizumab. The frequency of SAEs by body system has been comparable between the omalizumab and placebo-treated groups. The most frequently reported SAEs occurred in the digestive system (0.7% treated vs. 0.5% placebo), with appendicitis being the most frequently reported SAE (0.2% treated vs. 0.2% placebo). Other SAEs reported in more than 1 participant were: complication of study procedure (2 omalizumab, none placebo), myocardial infarction (2 omalizumab, none placebo), infectious mononucleosis (2 omalizumab, none placebo), and depression (2 omalizumab, none placebo). All other SAEs were reported in single participants in both the treatment groups. Adolescents have shown no increased rate of AEs or SAEs. The development of an immune response after administration of omalizumab cannot be excluded, but is not likely.

In previous studies more participants who received omalizumab reported cancer than those who received placebo or other treatments. The number of participants is too small to determine if there is a relationship between omalizumab and cancer. Similarly, some participants have experienced temporary urticaria (pale or reddened skin with itching or hives) when they receive their injections. In general, this side effect is rare, is mild to moderate in severity, and typically occurs with the first injection of omalizumab.

Anaphylactic shock is a possible allergic reaction to omalizumab. To date, no cases of anaphylactic shock have been associated with omalizumab.

1.4.2.2 Effects of Omalizumab on Platelets

In an effort to provide further safety data to support the use of higher doses of study drug compared with those studied in clinical trials and to support the use of omalizumab in children below the age of 6 years, dose-increasing toxicology studies were performed in young monkeys (aged 6-12 months) with omalizumab and rhumAb-E26. RhumAb-E26 is a compound similar to omalizumab.

With doses of omalizumab, low platelets were observed to occur rapidly after the first dose in young monkeys but had a slower onset in adult monkeys. No clinical signs related to the platelet drop were observed in the omalizumab-treated monkeys.

The monkeys treated with rhumAb-E26 (the similar compound) also showed severe reduced platelet levels. Blood samples were taken from the monkeys on Day 3 after a single dose of rhumAb-E26 was given. Decreased platelet counts and serious bleeding were observed in

the animals on Day 3, and 2 monkeys in this group died. The low platelet levels were reversible upon discontinuation of dosing in the surviving animals.

In contrast to these findings in young monkeys, omalizumab given to more than 1950 participants (6-75 years old) with asthma and/or seasonal allergic rhinitis in 16 clinical studies conducted over a 6-year period has not been associated with sustained decreases in platelet counts. The following nonserious bleeding-related adverse events were reported from the data from these 1950 participants:

- In early Phase II studies, when omalizumab was administered intravenously with a different formulation from that used in Phase III, bleeding-related adverse events (nosebleeds, heavy periods, bruising, and bleeding) were reported in 33 of the omalizumab-treated participants (8.4%) and 7 of the placebo-treated participants (4.3%). These bleeding-related adverse events were generally mild to moderate and of less than or equal to 1 day in duration. None of the bleeding-related adverse events were associated with a decreased platelet count.
- In the Phase III studies, bleeding-related adverse events (nosebleeds, blood in urine, bruising, and heavy periods) were reported in 29 of the omalizumab-treated participants (2.2%) and 14 of the placebo-treated participants (1.6%). None of the bleeding-related adverse events were associated with a decreased platelet count.

Additionally, 1 participant (enrolled in a long-term extension trial) with a low platelet count (95 x 10^3/μL) before starting omalizumab had platelet counts ranging from 25 x 10^3/μL to 98 x 10^3/μL (but no bleeding) while on omalizumab for more than 1 year. Although this 1 participant may have had a platelet disorder independent of omalizumab, the possibility that omalizumab contributed to this participant's low platelet counts were not ruled out.

As a measure of precaution, the study drug manufacturers have agreed with the Food and Drug Administration) to increase platelet monitoring for all participants receiving omalizumab during the course of this study.

1.4.3 Safety of Omalizumab and Immunotherapy

The safety findings for the use of the combination therapy with omalizumab and immunotherapy in one published study [32] (Section 1.3.3) are provided below.

A total of 221 SAR patients (birch and grass), randomized into 4 treatment arms (birch IT and omalizumab/placebo and grass IT and omalizumab/placebo), received IT plus anti-IgE or placebo for 24 weeks. The safety analysis in this study was performed on patients who received anti-IgE (n=114) and the rest of the participants (total N= 221). No SAE related to the study drug was reported in this study. Adverse event were reported by 80% the patients in 2 groups and most frequently reported AEs by both groups were cough, headache, and upper respiratory tract infection. Injection site reactions were common and occurred at similar rates in the 2 groups. Two cases of moderate urticaria were reported in the anti-IgE group that was deemed unrelated to the study drug. No cases of anaphylaxis or bleeding

were reported. Isolated reports of platelet counts <$150X10^9$/L (9 in the treatment group and 1 in the placebo group) returned to normal in subsequent visits while patients were receiving anti-IgE treatment.

No greater risk for the combination of omalizumab and IT is anticipated than using either modality alone. Since omalizumab should decrease serum free IgE levels, it is likely that the combination would be safer since the risk of developing an allergic reaction to RIT would be less in participants who have a markedly decreased IgE level. A number of safety measures are in place to help ensure that participants are not at risk; these measures include: frequent laboratory analyses and evaluations, frequent adverse event monitoring, the therapies will be administered in a clinical setting with adequate resuscitation capabilities and by trained personnel, the participants will be pre-treated by antihistamine starting at 24 hours before RIT, and a rescue antihistamine will be available for participants with significant symptoms of SAR.

Because clinical experiences with omalizumab and RIT as monotherapies have provided benefit to participants with SAR, it is anticipated that the combination therapy will provide similar, if not greater, benefit.

2 Objectives

The primary objectives of this study are to examine whether anti-IgE (omalizumab) given prior to rush IT (RIT) followed by 12 weeks of dual anti-IgE and IT is safer and more effective than RIT followed by IT alone in preventing the symptoms of ragweed-induced SAR. The secondary objectives of this study are to examine whether the combination of omalizumab with IT is safe and more effective than anti-IgE alone or placebo in preventing the symptoms of ragweed-induced SAR; to study the immunologic mechanisms of action associated with these therapies; and to study whether there is induction of tolerance after discontinuing these therapies as manifested by persistent inhibition of *in vivo* challenges and prolonged *in vitro* immunologic changes indicative of immune tolerance.

3 Study Design

3.1 Description

This clinical trial is a Phase II double blinded, placebo-controlled efficacy and safety evaluation of allergen immunotherapy co-administered with omalizumab in participants with ragweed seasonal allergy. This study will examine whether pre-treatment of participants with omalizumab followed by RIT, followed by dual therapy with omalizumab plus IT will be safer and more effective in preventing symptoms in ragweed-induced SAR versus omalizumab alone, IT alone or placebo. Omalizumab (or placebo) will be given starting 9 weeks prior to RIT, or placebo RIT, (either at 2 or 4-week intervals). After RIT, participants will receive either IT or placebo IT weekly and omalizumab (or placebo) every 2 or 4 weeks

for 12 weeks total. An initial follow-up period of 31 weeks, and an extended follow-up period of 28 weeks, will examine whether persistent immunologic and clinical tolerance was achieved. Clinical efficacy and safety will be examined during the ragweed season and throughout the study respectively, and potential immunological mechanisms invoked by both *in vitro* and *in vivo* studies will be examined. The *in vitro* tests will be done to examine whether omalizumab and IT are more effective at shifting the ragweed-induced immune response away from a Th2 paradigm, and towards an immune tolerant state. The *in vivo* studies will examine whether participants will have a prolonged inhibition of early and late-phase responses of ragweed-induced Allergy Skin Reaction Tests and nasal allergen challenges, which will be measured by acoustic rhinometry after therapy is discontinued, to show the persistence of immunologic and clinical changes.

SAR is an ideal model system to test new strategies to decrease pathologic immune responses (i.e. development of immune tolerance). There are well-defined clinical response parameters that can be measured, and in the case of ragweed-induced SAR, a time specific dosing strategy is feasible.

For risks associated with RIT, refer to Section 1.4.

3.1.1 Rush Immunotherapy

In order to temporally proximate the omalizumab injections with allergen IT, the IT dose will follow a rush immunotherapy protocol (RIT). RIT allows optimization of the timing of omalizumab administration before allergen IT. If a conventional allergen IT protocol were used, it would take up to 6 months to reach maintenance therapy. Therefore, it would be difficult to administer omalizumab in such a manner as to optimize the timing of the combination therapy. With RIT, by the end of one day, participants are close to the maintenance phase of IT and will have received the omalizumab in the appropriate timing for enhancement of safety, clinical efficacy, and induction of immune tolerance. The highest concentration used during RIT is 1:10 maintenance dilution; the maintenance dilution will contain approximately 40 µg/mL of *Amb a 1*. During the RIT, vital signs will be monitored every 30 minutes. RIT must be completed at least 3 weeks prior to the start of ragweed season.

3.1.2 Treatment Arms

It is anticipated that 168 participants will be needed to complete this double-blinded, parallel group, multicenter, placebo-controlled study. The participants will be randomized to 4 treatment groups as follows:

- Omalizumab pre-treatment, ragweed RIT, omalizumab and ragweed IT.
- Omalizumab pre-treatment, placebo RIT, omalizumab + placebo IT.
- Placebo omalizumab pre-treatment, ragweed RIT, placebo omalizumab + ragweed IT.
- Placebo omalizumab pre-treatment, placebo RIT, placebo omalizumab + placebo IT.

<table>
<tr><th colspan="2">Visit 0</th><th>Visits 1-3</th><th>Visit 4</th><th>Visits 5-16</th><th>Visits 17-20</th><th>Visits 21-28</th></tr>
<tr><th colspan="2">Wk -12 to -9</th><th>Wk -9, -5, -1</th><th>Wk 0</th><th>Wks 1 to 12</th><th>Wks 13-43</th><th>Wks 44-71</th></tr>
<tr><td rowspan="4">Screening</td><td rowspan="4">Randomization</td><td>Omalizumab Pre-treatment</td><td>RIT</td><td>Omalizumab +IT</td><td rowspan="4">Follow-up</td><td rowspan="4">Extended Follow-up</td></tr>
<tr><td>Omalizumab Pre-Treatment</td><td>Placebo RIT</td><td>Omalizumab + Placebo IT</td></tr>
<tr><td>Placebo Pre-treatment</td><td>RIT</td><td>Placebo Omalizumab + IT</td></tr>
<tr><td>Placebo Pre-Treatment</td><td>Placebo RIT</td><td>Placebo Omalizumab + Placebo IT</td></tr>
</table>

3.2 Study Endpoints

3.2.1 Primary Endpoint

The primary endpoint will be the average daily allergy severity score, which will be calculated from participants' 5 symptom scores (sneezing; rhinorrhea/runny nose; itchy nose, throat, and palate; itchy, watery eyes; and nasal congestion/stuffiness) during the 2003 ragweed_pollen season. Symptom scores are recorded twice daily (AM and PM). The ragweed pollen season begins when the ragweed pollen counts rise to 10 ragweed pollen grains/m^3/24 hours or above on two consecutive recorded days, and the ragweed pollen season ends when the ragweed pollen counts fall below 10 ragweed pollen grains/m^3/24 hours on two consecutive recorded days. The ragweed pollen season is from approximately August 15, 2003 to October 1, 2003, but varies among the sites. The sum of the individual symptom scores will be averaged over AM and PM to give a daily score. Each daily score will then be averaged to obtain one measure of the average daily allergy severity score for each participant.

3.2.2 Secondary Endpoints

The secondary variables are:

- The incidence and severity of adverse events;
- Number of days with rescue medication use during the 2003 ragweed season;
- Number of rescue medication capsules (fexofenadine HCl 60 mg) used during the 2003 ragweed season;
- Rhinoconjunctivitis QOL questionnaire (RQLQ) scores during the 2003 ragweed season;

- Daily AM allergy symptom scores during the 2003 ragweed season;
- Daily PM allergy symptom scores during the 2003 ragweed season; and
- Individual allergy symptom scores during the 2003 ragweed season.

The effects of Study Treatment on inducing an immune tolerant state will be explored through *in vivo* and *in vitro* immunologic tests. These exploratory endpoints will examine the effects of Study Treatment on shifting the ragweed-induced immune response toward a Th1 response and prolongation of the inhibition in early and late-phase responses to ragweed-induced allergy skin test and nasal allergen challenge (including nasal scraping). Please refer to Section 9 for a complete discussion of tolerance assays studies.

3.2.3 Tertiary Endpoints:

Tertiary endpoints were designed to evaluate whether the study treatment has any effects that persist during the 2004 ragweed season. These include:

- Average daily allergy scores during the 2004 ragweed season.
- Change in average daily allergy severity scores between the 2003 and 2004 ragweed seasons.
- Number of days with rescue medication use during the 2004 ragweed season;
- Number of rescue medication capsules (fexofenadine HCl 60 mg) used during the 2004 ragweed season;
- Rhinoconjunctivitis QOL questionnaire (RQLQ) scores during the 2004 ragweed season;
- Daily AM allergy symptom scores during the 2004 ragweed season;
- Daily PM allergy symptom scores during the 2004 ragweed season; and
- Individual allergy symptom scores during the 2004 ragweed season.
- Assessment of ragweed-specific IgG and IgE levels throughout the study.
- Assessment of other immunologic parameters as outlined in section 9.

3.3 Study Population

Approximately 168 study participants will be recruited based on the inclusion and exclusion criteria regardless of gender or ethnicity.

3.4 Screening, Randomization, and Enrollment

The study will be explained in lay language to each potential participant. The participant will sign an informed consent prior to any screening study procedures. Participants who are deemed qualified for the study will be randomized to 1 of 4 treatment arms and assigned a unique participant number. All activities performed in conjunction with this study will be performed in a blinded manner. An adequate number of participants will be screened to

ensure randomization of 168 patents. Based on previous experience, a total of 300 participants might need to be screened.

Although participants will be randomized to one of 4 treatment groups, the pharmacist or designee will observe 2 randomization episodes. In the first episode, participants will be randomized to either omalizumab or its placebo; in the second episode, the pharmacist or designee will be provided with randomization for either IT or its placebo.

3.5 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be chartered to review safety data and make recommendations regarding continuation, termination or modification to the study. Enrollment, safety and efficacy data will be submitted to the DSMB as appropriate.

3.6 Criteria for Discontinuation of Study Treatment

If, for any reason, Study Treatment is discontinued, the participants will be urged to continue the protocol-specified evaluations; they will be asked to return for all visits and complete all the clinical assessments without receiving Study Treatment. Study Treatment will be discontinued under the following conditions:

- If the participant becomes pregnant. In this case, Study Treatment must be immediately discontinued. Information about the participant, the participant's pregnancy, the outcome of the pregnancy, and the status of the infant at 8 to 12 weeks of age will be collected.
- If the participant develops systemic hypersensitivity reactions.
- If the participant does not receive three doses of omalizumab prior to RIT and does not receive the dose within the Visit Windows (Section 6.2).
- If a participant misses any post-RIT weekly immunotherapy dose and does not receive the dose within the Visit Windows (Section 6.4).
- In the Investigator's or Medical Monitor judgment it is in the participant's best interest.
- If any adverse event Grade 4 or higher is experienced by the participant that, by the assessment of the Investigator or Medical Monitor, is possibly, probably, or definitely related to Study Treatment or study participation.
- If the platelet counts are < 120 K on two consecutive occasions within 48 hours.
- The participant is not compliant with the study protocol.

3.7 Criteria for Terminating the Study/Stopping Rules

The rate and severity of adverse events will be closely monitored by Investigators and Medical Monitors and reported to regulatory authorities per protocol and enforced regulations. The DSMB, Investigators, Medical Monitors, or participating Institutional Review Boards (IRBs) shall be immediately informed should there be any life threatening,

disability-inducing, or unexpected serious adverse event associated with the use of Study Treatment.

During the RIT period, any adverse events will be closely monitored and followed appropriately; Investigators, the ITN Medical Monitor, the NIAID Medical Officer, and as appropriate, other involved authorities will be informed of these adverse events and will remain in daily communication through conference calls and/or electronic or personal communications.

The enrollment will be stopped, and no further Study Treatment will be administered to any participant at any study site if any of the following occurs:

- Any death, due to possible participation in the study, occurs in the study. The study will be put on temporary hold until it is determined that the death was not due to study participation. If it is determined that the death was due to participation in the study, the resumption of the study will be contingent upon a formal review by the DSMB and ITN.
- At the request of the DSMB for safety of the participants.

The resumption of the Study Treatment will be contingent upon favorable decisions of Investigators, DSMB, ITN, NIAID, and any regulatory authorities after careful review of the study data.

4 Selection and Withdrawal of Participants

4.1 Inclusion Criteria

- Able to comprehend and grant a witnessed, written informed consent prior to any study procedures.
- Male or female 18 to 50 years of age.
- Female participants of child bearing age must have a negative urine pregnancy test at Visit -01 and a negative urine pregnancy test at subsequent visits. In addition, female participants must be using a medically acceptable form of birth control.
- History of seasonal allergic rhinitis for at least 2 years with symptoms during the ragweed pollen season requiring pharmacotherapy.
- A positive skin test by prick method to ragweed pollen at Visit -01. A positive skin prick test will be defined as a ragweed pollen-induced wheal ≥ 3 mm larger in diameter than diluent control (measurements will be made 15-20 minutes after application).
- Must be capable of faithfully completing the diary and of attending regularly scheduled study visits.
- Should intend to remain in the ragweed pollen area from August 15 through September 30. Any travel outside of the pollen area should be noted in the source documents. Participants who travel outside of the ragweed pollen area for prolonged periods of time should be evaluated by the Investigator as to their suitability to continue in this study.
- Willing to avoid prohibited medications for the periods indicated in the protocol.

- Participants must meet pretrial eligibility requirements for trial enrollment (acceptable medical history, physical examination results, normal electrocardiogram and acceptable laboratory test results).
- Participants must have a baseline serum IgE level $\geq$ 10 and $\leq$ 700 IU/mL.

4.2 Exclusion Criteria

- Participants < 30 kg or > 120 kg.
- Participants who are pregnant or lactating.
- Participants with a history of severe anaphylactoid or anaphylactic reaction(s).
- Participants with a history of immunotherapy within the past 10 years, if received one full year of immunotherapy, or within the past 5 years if received less than one year of immunotherapy.
- Participants with known hypersensitivity to trial rescue medication (fexofenadine HCl).
- Participants taking beta-adrenergic antagonists in any form.
- Participants taking ophthalmologic medication for allergic symptoms.
- Participants with clinically significant perennial rhinitis that would interfere in assessment of ragweed-induced seasonal allergic rhinitis symptoms.
- Presence of a severely deviated nasal septum, septal perforation, structural nasal defect or large nasal polyps causing obstruction.
- History of an upper respiratory or sinus infection requiring treatment with an antibiotic within 2 weeks prior to Screening Visits.
- Documented evidence of acute or significant chronic sinusitis, as determined by the Investigator.
- Asthma (either history of, abnormal spirometry [FEV_1 < 80% predicted], or use of asthma medications as specified in Section 5.10).
- Chronic or intermittent use of inhaled, oral, intramuscular, or intravenous corticosteroids; or chronic or intermittent use of topical corticosteroids within 4 weeks of Screening Visits.
- Chronic use of medications (e.g., tricyclic antidepressants) that would affect assessment of the effectiveness of the study medication.
- Rhinitis medicamentosa.
- History or presence of significant renal, hepatic, neurologic, cardiovascular, hematologic, metabolic, cerebrovascular, respiratory, gastrointestinal, or other significant medical condition, including autoimmune or collagen vascular disorders, aside from organ-specific autoimmune disease limited to the thyroid that in the Investigator's opinion could interfere with the study or require medical treatment that would interfere with the study.
- History of cancer other than basal cell carcinoma of the skin.
- History within the past year of excessive alcohol intake or drug addiction.
- Current smokers, greater than 10 pack year history, or participants who quit smoking less than one year prior to Screening.
- Use of any prohibited concomitant medications during the washout period
- Participants currently undergoing immunotherapy.

- Participants with clinically significant abnormality on 12-lead electrocardiogram (ECG) on screening visit.
- Treatment with an experimental, non-approved drug, or investigational drug within the past 30 days.
- Participants with a history of noncompliance to medical regimens and participants who are considered potentially unreliable.
- Previous treatment with a monoclonal antibody for any reason including anti-IgE in any form (e.g., omalizumab).
- Participants with known hypersensitivity to trial drug ingredients (i.e., sucrose, histidine, polysorbate 20) or related drugs (i.e., monoclonal antibody, polyclonal gammaglobulin).

4.3 Participant Withdrawal Criteria

Participants will be informed that they are free to withdraw from the study at any time without jeopardizing their relationship with their health care providers

Participants who stop receiving Study Treatment will be urged to continue the protocol specified evaluations; they will be asked to return for all visits and complete all the clinical assessments. All data normally collected at the completion of the study must be collected either at the time of the participant's early termination or on or before the scheduled study close-out date.

Participants who experience adverse events will be followed until all significant changes have returned to baseline or stabilized. All study termination reasons will be recorded on the relevant CRF.

Participants who receive any study-related treatment will be included in the safety analysis of the data. All randomized patients will be included in the intent-to-treat analysis of the data, regardless of whether they subsequently withdraw from the study.

4.3.1 Premature Termination from the Study

Participants will be withdrawn from the Study Treatment and will make no additional visits only if they, or their guardians, withdraw consent.

4.3.2 Modification or Temporary Discontinuation of Study Medication

If a participant develops local reactions to the maintenance immunotherapy dose after the rush immunotherapy, no dosage adjustment will be made. If a participant experiences a systemic reaction requiring epinephrine, he/she will receive no further Study Treatment.

If a participant develops any adverse event that warrants temporary discontinuation of Study Treatment as determined by the Investigator, the Medical Monitors will be informed. The

Investigator, in consultation with Medical Monitor and, as appropriate, the Data and Safety Monitoring Board (DSMB), will try to resume Study Treatment if deemed appropriate.

In cases that the Study Treatment is not resumed, the participant will remain in the study and complete all the study-related visits and procedure according to the follow-up visit schedule, without receiving the Study Treatment and will be included in the intent-to-treat analysis. All temporary discontinuation of Study Treatment will recorded and reported as appropriate.

4.3.3 Replacement of Participants who Discontinue Study Treatment or who Prematurely Terminate from the Study

Participants withdrawn from the study before receiving any Study Treatment will be replaced. Those who received Study Treatment to any extent shall not be replaced and will be included in the intent-to-treat analysis.

5 Study Treatments

5.1 Omalizumab

5.1.1 Dosing Regimen

Exploratory analyses of a variety of clinical response data from several studies of omalizumab in allergic asthma and SAR have suggested clinical benefit at serum free IgE concentrations < 10–30 ng/mL. A minimum equivalent dose of 0.016 mg/kg/IgE (IU/mL) every 4 weeks has been determined to reduce serum free IgE concentration to < 10-30 ng/mL. The minimum doses and dosing regimen necessary to achieve this targeted free IgE suppression have been presented in the table in Appendix 2. This table is a simplified dosing strategy. The individualized dose scheme (mg/kg/IgE [IU/mL]) has been modified to group individuals into tiers in which each patient receives at least the proposed minimum effective dose.

Appendix 2 contains the dosing regimen table for a 4-week dosing interval to deliver a minimum dose of 0.016 mg/kg/IgE (IU/mL). Each participant's required total dose will be first determined according to the 4-week interval dosing regimen table (Appendix 2). If the participant's dose is ≤ 300 mg, omalizumab will be administered once every 4 weeks. If the participant's required dose, according to this table, is > 300 mg per 4-week interval, the dose will be split into 2 equal doses and administered every 2 weeks; this will deliver a minimum dose of 0.008 mg/kg/IgE (IU/mL) per 2-week dosing schedule. Therefore, participants will either receive 6 (3 pre-treatment, 3 post-RIT) SC injections of omalizumab, or placebo, if they require ≤ 300 mg, at Weeks -9, -5, -1, 3, 7, and 11, or 11 (5 pre-treatment, 6 post-RIT) SC injections of half doses of omalizumab or placebo, if they require > 300 mg per 4-week dosing interval, at Weeks -9, -7, -5, -3, -1, 1, 3, 5, 7, 9, and 11.

5.1.2 Formulation, Packaging, and Labeling

Omalizumab is supplied by Genentech, Inc. (So. San Francisco, CA) as a sterile, white to off-white, preservative-free, lyophilized powder contained in a single-use vial. Each vial contains 202.5 mg of omalizumab, sucrose, L-histidine, L-histidine hydrochloride monohydrate, and polysorbate 20.

5.1.3 Preparation, Administration and Dosage

Omalizumab is supplied as a sterile, freeze-dried preparation that can be reconstituted to a final concentration of 125 mg/mL. Each vial contains approximately 202.5 mg of omalizumab and is designed to deliver 150 mg of omalizumab after reconstitution with 1.3 mL of sterile water for injection, USP. Only 1.2 mL of the reconstituted solution will be drawn from the vial to deliver 150 mg of the active substance. After reconstitution, the contents are gently swirled for 30 seconds, and then left to solubilize. The lyophilized product typically takes 10–20 minutes to dissolve completely; some vials may require more than 20 minutes. The vial should not be inverted and the formulation does not contain a preservative. It is therefore to be used for a single-dose administration only. For complete discussion of reconstitution procedures and storage, please refer to Appendix 2.

5.2 Immunotherapy

Immunotherapy consisting of escalating doses of short ragweed extract will be administered in a rush IT protocol (Appendix 3) in which participants will receive up to 0.3 mL of a 1:10 maintenance dilution of ragweed according to the table in Appendix 3; the maintenance dilution contains 40 μg/mL of *Amb a 1*. RIT must be completed at least 3 weeks prior to the start of ragweed season. Participants will then receive weekly IT dosing for a total of 12 weeks according to the table provided in Appendix 3. Participants must achieve a minimum of 0.15 mL of the 1:1 maintenance dilution by the third week. If tolerated, ragweed immunotherapy will be increased to 0.3 mL of 1:1 maintenance dilution. If large local reactions that are not tolerated by the participant are noted with doses greater than 0.15 mL, the investigator, at his discretion, may lower the next dose to the previously tolerated dose. Thus, the highest tolerated dose, either 0.15, 0.20, or 0.3 mL, will be administered. Once the dose is reduced, it cannot be increased. If participants cannot tolerate a minimum dose of 0.15 mL of maintenance dilution, the Study Treatment must be discontinued.

To increase safety, participants will receive 180 mg of fexofenadine 24 hours and 1 hour prior to RIT administration.

The use of fexofenadine 60 mg prior to weekly IT injections will be permitted at the discretion of the investigator.

5.3 Placebo

The placebo for omalizumab will contain the excipients and diluents of the omalizumab drug product and is supplied by Genentech, Inc.

The placebo for immunotherapy will contain the diluents and histamine as supplied by ITN/NIAID and partially prepared by the pharmacist or designee at each site.

5.4 Drug Accountability

Under federal regulations (21CFR 312.62) an Investigator is required to maintain adequate records of the disposition of the investigational agent, including dates, quantity of drug received, to whom dispensed (participant by participant accounting), and accounts of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant and should contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.5 Disposition of Unused Drug

Unused portions of prepared omalizumab will be discarded. Any unused and intact omalizumab will be destroyed at sites as per specific instructions provided by the manufacturer (Genentech, Inc.) to each site.

5.6 Assessment of Compliance with Study Medication

Omalizumab will be administered via SC injection by medical staff in a clinical setting; compliance therefore, will be monitored by the medical staff and documented on the case report form.

5.7 Study Visit Windows

5.7.1 Pre-treatment

If a participant misses 1 dose of pre-treatment they will have to receive the dose within 3 days of the missed injection date. If the participant does not receive 3 doses of omalizumab prior to rush immunotherapy, they will not receive further Study Treatment.

5.7.2 Treatment

If a participant misses any post-RIT weekly immunotherapy dose, they should receive the dose within 3 days of the scheduled date. If the participant does not receive the dose, no further Study Treatment will be administered.

5.7.3 Follow-up Phase and Extended Follow-up

If a participant misses any follow-up visit, they should be seen within 5 days of the scheduled visit.

5.8 Rescue Medication

Fexofenadine HCl 60 mg is the rescue medication and will be available for use providing the participant has sufficient and documented symptoms. Participants will be dispensed fexofenadine HCl 60 mg at each study visit starting at Visit 01. During the pre-treatment phase and in between ragweed seasons, pill counts at each visit will be used to record the number of rescue medication used. During the IT treatment phase (post RIT) and during the 2003 and 2004 ragweed seasons, the use of rescue medication will be recorded on the diary cards and verified by the pill counts. Participants must have a rating of at least moderate to severe on at least 2 individual allergy symptoms in order to use the rescue medication during the study. Participants are required to avoid rescue medication 72 hours prior to nasal allergen challenge and/or any skin tests. Patients should avoid the use of fexofenadine the day of scheduled visits.

The use of this rescue medication should help prevent participant dropouts. Fexofenadine was chosen as rescue therapy because it has a very good safety profile and is relatively devoid of impairment and somnolence side effects so that no additional risks will be involved for the participants.[(44)]

Note: Visit 20 rescue medication cannot be dispensed until the participant has signed the addendum to the informed consent. Therefore, Visit 20 rescue medication may actually be dispensed sometime between Visits 20 and 21.

5.9 Concomitant Medications

All medications used by participants at the time of screening and throughout the study will be recorded and reported in relevant CRF.

5.10 Prohibited Medications

The medications listed below are prohibited during the washout period (before screening) and throughout the study. Any use of such medications during the trial will be recorded and reported as protocol deviations. Herbal supplements will be prohibited throughout the study at the discretion of the Investigator if it is believed that they might influence study assessments.

Note: Between visits 20 and 21 only, participants may use prohibited medications provided that the wash out periods have been observed before visit 21. Therefore, the use of these medications will not be considered protocol violations during that time, but they must be recorded on the Concomitant Medication CRF.

Prohibited Medication	**Washout period (time prohibited prior to Screening or Visit 21)**
Allergic ophthalmologic medications	1 week
Intranasal Cromolyn	2 weeks
Intranasal or Systemic Decongestants	3 days
Intranasal or Oral Antihistamines (except rescue antihistamine as per protocol)	1 week
Intranasal Anticholinergics	1 week
Tricyclic Antidepressants	1 week
Corticosteroids, of all forms	4 weeks
Beta Adrenergic Antagonists	1 week
Leukotriene Modifiers	1 week
Investigational Drugs	4 weeks (or a minimum of 3 half-lives)
Vaccinations	4 weeks

6 Study Procedures

The study is divided into 6 periods:

1. Screening and baseline (3 weeks);
2. Omalizumab pre-treatment (9 weeks);
3. Rush immunotherapy (1 day);
4. Omalizumab/placebo and immunotherapy/placebo (12 weeks); and
5. Follow-up (31 weeks).
6. Extended follow-up phase for 2004 ragweed season (28 weeks)

The Procedures and Assessments during these 5 periods are described below and are tabulated in Appendix 4.

6.1 Screening

During screening, participant's eligibility will be assessed. All participants must sign an informed consent prior to any protocol screening procedures. There will be 2 Screening Visits; procedures at each visit are described below and in Appendix 1.

6.1.1 Screening Visit -01

- Informed consent.
- Medical history.
- Ragweed pollen history.
- Medication history (for the previous 3 months).
- Vital signs (including temperature, respiratory rate, blood pressure, height, weight, and pulse).
- Complete physical examination (including assessments of general appearance, hair and skin, lymphatics, head, eyes, nose, throat, neck, lungs, cardiovascular, abdomen, musculoskeletal, neurological, mental status).
- Allergy skin test consisting of:
    - Prick tests placed approximately 5 cm apart using a separate needle for each test.
    - Positive control (histamine base 1 mg/mL) and negative control (extract diluent).
    - Ragweed extract at 1:20 (weight/volume) in 50% glycerin.
- Allergy skin test will be read after 15-20 minutes.
- Total IgE level
- Hematology (including hemoglobin, hematocrit, RBCs, MCV, MCH, MCHC, white blood cells, WBC differential, platelet count).
- Blood chemistry (including BUN, creatinine, glucose, uric acid, total protein, albumin, triglycerides, cholesterol, CK, potassium, sodium, calcium, total bilirubin, alkaline phosphatase, ALT, AST, gamma GT, LDH).
- Urinalysis (including appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, blood, microscopic evaluation).
- Urine pregnancy test.
- Pulmonary function test (FEV_1, FVC and FEV_1/FVC)
- Twelve-lead ECG.
- Confirmation of eligibility.

6.1.2 Screening Visit 00

Continued eligibility will be assessed along with baseline responses to allergen challenges Participants are instructed to avoid any rescue medication 72 hours prior to nasal allergen challenge and/or skin tests.

- Vital signs.
- Interim medical history.
- Interim physical exam.
- Allergy skin reactions test.
- Nasal allergen challenge (including nasal scraping).
- Nasal exhaled nitric oxide (NO).
- Collect serum for human anti-human antibody (HAHA) testing.
- Urine pregnancy test.
- Confirmation of continued eligibility.
- Immune tolerance lab testing.
- Adverse event assessment.

6.2 Omalizumab Pre-Treatment

During the pre-treatment period, participants will receive SC injections of omalizumab or matching placebo at each of the 3 visits. Those requiring > 300 mg of omalizumab, will receive the drug in 2 equal split doses, 2 weeks apart. Therefore, these participants will require interim visits to receive extra injections at Weeks -7 and -3 (Section 6.5). Study visits during this omalizumab pre-treatment period should occur within ± 3 days of the targeted date. During the 3 visits in this period, the following will be performed:

- Vital signs (Visits 1 - 3).
- Interim medical history (Visits 1 - 3).
- Interim physical exam (Visits 1 - 3).
- Hematology (including hemoglobin, hematocrit, RBCs, MCV, MCH, MCHC, white blood cells, WBC differential, platelet count) (Visits 1 - 3).
- Blood chemistry (including BUN, creatinine, glucose, uric acid, total protein, albumin, triglycerides, cholesterol, CK, potassium, sodium, calcium, total bilirubin, alkaline phosphatase, ALT, AST, gamma GT, LDH) (Visits 1 - 3).
- Urinalysis (including appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, blood, microscopic evaluation) (Visits 1 - 3).
- Urine pregnancy test (Visits 1 - 3).
- Confirmation of continued eligibility (Visits 1 - 3).
- Quality of Life questionnaire (Visit 1).
- Randomization to omalizumab or placebo (Visit 1).
- Omalizumab or placebo treatment (Visits 1 - 3). Those requiring > 300 mg omalizumab, will receive extra injections at Weeks -7 and -3 (Interim Visits Section 6.5).
- Dispense rescue medication (Visits 1-3).
- Record rescue medication use – pill count (Visits 2 – 3).

- Adverse event assessment (Visits 1 - 3).

6.3 Rush Immunotherapy

During RIT, participants will receive escalating doses of ragweed extract, or matching placebo, according to the table in Appendix 3. All participants will have multiple safety evaluations prior to each injection, recording any and all adverse events. Participants randomized to receive placebo RIT will receive escalating concentrations of histamine. Participants will receive 180 mg of fexofenadine 24 hours and 1 hour prior to RIT administration. The study visit for RIT and associated assessments should occur within ± 3 days of the targeted visit date.

- Vital signs every 30 minutes during RIT.
- Interim medical history.
- Interim physical exam.
- Collect serum for human anti-human antibody (HAHA) testing.
- Confirmation of continued eligibility.
- Immune tolerance lab testing (see Section 9). Blood sample for these tests can be drawn up to 72 hours before a scheduled visit.
- Record medication use- pill count
- Dispense rescue medication.
- Issue participant diary cards.
- Rush immunotherapy.
- Adverse event assessment.

6.4 Omalizumab or Placebo and Immunotherapy or Placebo

During this period, participants will receive either omalizumab or placebo every 2 or 4 weeks with concomitant weekly injections of ragweed IT or placebo. During this period of the study, visits occur on a weekly basis (within ± 3 days of the target date). Participants are instructed to avoid any rescue medication 72 hours prior to nasal allergen challenge and skin tests. However, the use of fexofenadine 60 mg prior to each IT injection will be permitted at the discretion of the investigator. **Note:** Visit 17 must occur at least 1 week (but no later than 4 weeks) after the end of ragweed season as determined by 2 consecutive days with recorded pollen counts of less than 10 ragweed pollen grains/m^3/24 hours. Participants will be provided enough diary cards at visit 16 to cover any possible extended period between visits 16 and 17. Visit 18 will occur 6 weeks after the scheduled visit 17.

Procedures and assessments during this period are:

- Vital signs (Visits 5 – 16).
- Interim medical history (Visits 5 – 8, 11, 15, and 16)
- Interim physical exam (Visits 7, 11, and 15)
- Allergy skin reactions test (Visit 9)

- Exhaled nasal NO (Visits 5, 7, 9, 11, 13, and 15)
- Hematology (including hemoglobin, hematocrit, RBCs, MCV, MCH, MCHC, white blood cells, WBC differential, platelet count) (Visits 7, 11, and 15)
- Blood chemistry (including BUN, creatinine, glucose, uric acid, total protein, albumin, triglycerides, cholesterol, CK, potassium, sodium, calcium, total bilirubin, alkaline phosphatase, ALT, AST, gamma GT, LDH) (Visits 7, 11, and 15)
- Collect serum for human anti-human antibody (HAHA) testing (Visits 5, 9, and 13)
- Urinalysis (including appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, blood, microscopic evaluation) (Visits 7, 11, and 15)
- Urine pregnancy test (Visits 7, 11, and 15). Those requiring > 300 mg omalizumab will have urine pregnancy tests at Visits 5, 9, and 13.
- Confirmation of continued eligibility (Visits 5 – 16)
- Immune tolerance lab testing (see Section 9) (Visits 5, 9, and 13)
- Dispense rescue medication (Visits 5 – 16)
- Record rescue medication use – pill count (Visits 6 – 16)
- Issue participant diary cards (Visits 5 – 16)
- Collect and review diary cards (Visits 6 - 16)
- Quality of life questionnaire (Visits 5, 8, 11, and 14)
- Omalizumab/placebo treatment (Visits 7, 11, and 15). Those requiring > 300 mg omalizumab, will receive omalizumab at Visits 5, 7, 9, 11, 13, and 15). Omalizumab will be administered 60 minutes after the IT injection.
- Immunotherapy (Visits 5 – 16) will be administered 60 minutes before omalizumab injections.
- Adverse event assessment (Visits 5 – 16)

6.5 Interim Visits

As described in Section 5.1.1 and depicted in the dosing regimen table in Appendix 2, the dose of omalizumab will be determined by the body weight and level of the baseline IgE. Each participant's required dose per 4-week interval will be determined using the dosing regimen table (Appendix 2). Participants who require > 300 mg of omalizumab per this table, will have to receive the drug in two equally split doses, administered 2 weeks apart. Therefore, these participants will have to be seen for 2 extra interim visits at Weeks -7 and -3; other extra omalizumab injections will occur during the weekly IT visits. The following will be assessed during the interim visits:

- Urine pregnancy test.
- Vital signs.
- Adverse event assessment.

6.6 Unscheduled Visits

Unscheduled visits include any visits in addition to the scheduled visits that the patient must make to comply with the required study procedures. For example, if a patient needs to repeat an RIT injection or an IT injection because of a systemic reaction that did not require epinephrine, he/she can do so at an unscheduled visit (USV). During the USV, the patient will also be monitored for vital signs and adverse events. If the patient receives omalizumab, a urine pregnancy test must be performed first.

6.7 Primary Follow-up

Participants will be followed through 43 weeks post-RIT. Four follow-up visits are scheduled; these should occur within ± 5 days of the scheduled day. Participants are instructed to avoid any rescue medication 72 hours prior to nasal allergen challenge and skin tests. **Note:** Visit 17 must occur at least 1 week (but no later than 4 weeks) after the end of ragweed season as determined by 2 consecutive days with recorded pollen counts of less than 10 ragweed pollen grains/m^3/24 hours. Participants will be provided enough diary cards at visit 16 to cover any possible extended period between visits 16 and 17. Visit 18 will occur 6 weeks after the scheduled visit 17. During this period, safety and efficacy will be assessed.

- Vital signs (Visits 17 – 20).
- Interim medical history (Visits 17, 19, and 20).
- Interim physical exam (Visits 17 and 19).
- Complete physical examination (including assessments of general appearance, hair and skin, lymphatics, head, eyes, nose, throat, neck, lungs, cardiovascular, abdomen, musculoskeletal, neurological, mental status) (Visit 20).
- Allergy skin reactions test (Visits 17 – 20).
- Nasal allergen challenge (including nasal scraping) (Visits 17 – 20).
- Nasal exhaled NO (Visit 17).
- Hematology (including hemoglobin, hematocrit, RBCs, MCV, MCH, MCHC, white blood cells, WBC differential, platelet count) (Visits 17 and 20).
- Blood chemistry (including BUN, creatinine, glucose, uric acid, total protein, albumin, triglycerides, cholesterol, CK, potassium, sodium, calcium, total bilirubin, alkaline phosphatase, ALT, AST, gamma GT, LDH) (Visits 17 and 20).
- Collect serum for human anti-human antibody testing (Visits 17 - 20).
- Urinalysis (including appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, blood, microscopic evaluation) (Visits 17 and 20).
- Urine pregnancy test (Visits 17, 19, and 20).
- 12-lead ECG (Visit 17).
- Confirmation of continued eligibility (Visits 17 - 20).
- Immune tolerance lab testing (see Section 9) (Visits 17 – 20).
- Record rescue medication use – pill count (Visit 17).
- Collect and review diary cards (Visit 17).
- Quality of life questionnaire (Visit 17).

- Adverse event assessment (Visits 17 – 20).

6.8 Extended Follow-up Phase (2004 Ragweed Season)

Study participants who have received all required study treatments during the treatment phase and who are still enrolled at the end of the follow-up phase will be considered for the extended follow-up phase. These participants will be asked to sign a consent addendum at Visit 20 or the soonest convenient time thereafter. Participants will then be evaluated starting approximately one month before the start of the 2004 ragweed season through the post-ragweed season.

The purpose of this extended follow-up phase is to examine if study treatments received in 2003 have any effects that persist during the 2004 ragweed season. This will be assessed through tertiary endpoints outlined in section 3.2.3. No treatment will be administered during this period. Please refer to section 5.10 for the use of prohibited medication during this period. Participants and physicians will remain blinded to the original treatment assignment.

Total duration of the extended follow-up phase is approximately 28 weeks. During the extended follow-up phase, participants will be issued diary cards to record their allergy symptoms for 12 weeks. Participants must follow all the stipulations and restrictions imposed by this protocol. Visit 21 will be scheduled no later than 2 weeks prior to the ragweed season (~early July 2004). Visits 22 through 27 will then be scheduled every 2 weeks. Visit 28 (the close-out visit) will be scheduled no sooner than 2 weeks post-ragweed season. The following table should be used as a reference when scheduling extended follow-up visits:

Table 1: Extended phase visit dates

Visit Number	Suggested Visit Dates (+/- 5 days)
21	July 12-17
22	July 26-31
23	August 9-14
24	August 23-28
25	September 6-11
26	September 20-15
27	October 4-9
28	No sooner than 2 wks post-ragweed season

Procedures and assessments during the extended follow-up phase include the following. (Please refer to Appendix 4.)

- Interim Medical History (Visits 21-28)

- Interim Physical Exam (Visits 21 and 28)
- Vital signs (Visits 21 and 28)
- Allergy Skin Reactions Test (Visits 21 and 28)
- Nasal allergen challenge test including nasal scraping (Visits 21 and 28- for a select group of patients)
- Confirmation of Eligibility (Visits 21-28)
- Dispense rescue medication (Visits 21-27)
- Record rescue medication use – pill count (Visits 22-28)
- Issue diary cards (Visits 21-26)
- Collect and review diary cards (Visits 22-27)
- Quality of Life Questionnaire (Visits 23, 25, and 27)
- Adverse Event Assessment (Visits 21-28)
- Urine pregnancy test (Visits 23 and 28)
- Immune tolerance lab testing (Visits 21, 24 and 28)

7 Assessments of Safety and Efficacy

7.1 Safety

Safety will be assessed by the incidence and severity of local and systemic adverse events, including laboratory assessments at anytime during the treatment and follow-up periods.

7.2 Efficacy

Efficacy will be determined by the participant's assessment of severity of SAR-related symptoms, the quantity of rescue medication consumed, the scores obtained from the quality of life questionnaire, and the *in vivo* and *in vitro* tolerance assessments.

7.3 Tolerance Induction

Tolerance induction will be judged by whether therapy results in a sustained reduction of *in vivo* responses to allergen, including early and late-phase skin tests, and nasal challenges, as well as *in vitro* laboratory parameters (see Section 9) indicative of antigen-specific tolerance during the follow-up phase after study treatment has been discontinued as well as during the extended follow phase (see section 6.8). Data on *in vivo* and *in vitro* responses will be collected throughout the study.

7.3.3 *In Vivo* Responses

7.3.3.1 Early and Late Phase Skin Reactions

Note: The short ragweed extract provided in this study contains 546 μg/mL *Amb a 1* (during the 2003 treatment and follow up phase) and 368 mcg/ml Amb a1 (during the 2004 extended follow up phase). The pharmacist or designee is required to prepare a diluted solution of the original ragweed extract vial, so that this solution contains approximately 273 μg/mL *Amb a 1* (during the treatment and follow up phase) and 283 μg/mL *Amb a 1* (during the extended follow up phase) and then follow the procedures below. This diluted solution is considered the "1:10" vial for the intradermal (intracutaneous) skin test.

Allergy skin testing will be performed with standardized ragweed extract in Visits 00, 9, 17, 18, 19, 20 and during the extended follow-up phase. Any antihistamine, including the rescue medication, is prohibited 72 hours prior to skin test or nasal allergen challenge. Approximately 0.02 mL of ten-fold dilutions of 1:10,000,000, 1:1,000,000, 1:100,000 and 1:10,000 will be injected intracutaneously. If the subject tolerates the four previous dilutions, a dilution of 1:1000 will be administered intracutaneously. The highest concentration (1:1000 dilution), that might be used, will contain approximately 2.73 to 2.83 μg/mL of *Amb a 1*. Wheal and erythema responses will be measured as the average of the sum of longest diameter and longest perpendicular diameter measured at 15 minutes. The mean values for the wheals will be summed to obtain a mean value for all concentrations. Late phase responses will be examined 16 to 24 hours after the injections and analyzed in an identical fashion as those outlined for the immediate measurement at 15 minutes. Mean values will be recorded for the positive control (histamine base 1 mg per mL) and negative control (extract diluent) injections in the same manner.

The skin test reactions will be recorded by outlining the wheal and erythema with a fine tip black pen and placing transparent tape over the outlying skin reaction. The tape will then be lifted along with the adherent ink off the skin and placed into the source documents

7.3.3.2 Nasal allergen challenge (including nasal scraping(s))

Note: The short ragweed extract provided in this study contains 546 μg/mL *Amb a 1* (during the 2003 treatment and follow up phase) and 368 mcg/ml Amb a1 (during the 2004 extended follow up phase). The pharmacist or designee is required to prepare a diluted solution of the original ragweed extract vial, so that this solution contains approximately 273 μg/mL *Amb a 1* (during the treatment and follow up phase) and 283 μg/mL *Amb a 1* (during the extended follow up phase) and then follow the procedures below. This diluted solution is considered the "1:10" vial for the intradermal (intracutaneous) skin test.

All participants enrolled in the study will undergo nasal allergen challenge with ragweed. The nasal allergen challenge will comprise four periods: (1) baseline; (2) challenge; (3) immediate phase reaction; and (4) late phase reactions measured 16 to 24 hours after

challenge. Any antihistamine, including the rescue medication, is prohibited 72 hours prior to skin test or nasal allergen challenge.

Participants are instructed to avoid any rescue medication 72 hours prior to nasal allergen challenge. Nasal allergen challenges will be performed in Visits 00, 17, 18, 19 and 20, and in a selected group of participants at Visits 21 and 28. This test must occur before skin testing on the same day, since allergy skin testing involves multiple injections of ragweed, which might influence nasal responses. However, the opposite is not true. At baseline, an acoustic rhinogram will be recorded, pulmonary function performed and nasal symptoms assessed. Nasal symptoms include sneezing, itching, and sinus pressure will be assessed on a 0 (none), 1 (mild), 2 (moderate), and 3 (severe) scale. Vehicle control (saline) will be administered to the nose via nasal spray pump. Two sprays will be administered to each nostril (2 sprays = 0.180 mL) for three times. Ten minutes after the third saline administration, acoustic rhinometry and pulmonary function will be performed and nasal symptoms will be assessed. This will be used as baseline. After a third saline control, nasal scrapings will be taken from the inferior and medial turbinates anteriorly using a rhinoprobe. Rhinoprobe samples will be analyzed for cell differentials for inflammatory cells (examining 100 inflammatory cells) and real-time PCR examining for: IL-4, IL-5, IL-13, IL-10, IL-9, TGFβ, IFN-γ, MIP-1α, MIP-1β, and TARC.

Immediately thereafter, the challenge phase will begin which will consist of administering increasing concentrations of ragweed at 1;1,000,000, 1:100,000, 1:10,000, 1:1000, 1:100, and 1:10 (the 1:10 contains approximately 273-283 μg/mL of *Amb a 1*). Ten minutes after each administration of an allergy concentration, an acoustic rhinogram will be recorded, pulmonary function performed and nasal symptoms assessed (sneezing, itching, and sinus pressure). The participant is instructed to blow nose before rhinometry. The challenge will continue until: 1) There is a 30% decrease in nasal volume as determined by acoustic rhinometry, 2) the maximum concentration is reached, or 3) A 20% decrease in FEV_1 is reached. After a 30% decrease in nasal volume is observed, a repeat rhinogram will be performed at 30 minutes after the allergen challenge with recording of nasal volume.

The provocative dose to elicit a 30% decrease in nasal volume (PD_{30}) will be calculated by interpolating the 30% decrease in total nasal volume between ΣA and ΣB, where ΣA=the summation of all doses below the 30% threshold; and where ΣB = ΣA + the dose used to cross the 30% threshold. "Dose" is calculated by multiplying the *AMB a 1* concentration for each stage by 0.36 (0.36 represents four sprays of 90 microliters to equal 360 microliters of solution administered to the patient at each timepoint.)

The PD30 will be diluted by 4 in order to allow for 4 sprays of 90 microliters to be administered during the challenge.

Once participants have a nasal allergen challenge PD 30 defined, they will receive that dose at every subsequent visit. If no PD 30 is achieved the 1:10 dilution will be administered at subsequent visits .The investigators will assess participants to determine if they have a decrease in nasal allergen challenge-induced nasal volume subsequent to treatment.

Participants will return in 16 to 24 hours for a repeat acoustic rhinometry measure. Scrapings using a rhinoprobe will be taken from the left nares at 16 to 24 hours for a cell differential and RT-PCR as outlined above.

7.3.3.3 Nasal Nitric Oxide

Exhaled NO is a measure of airway inflammation.[45] Nasal NO will be assessed periodically throughout the study, in Visits, 00, 5, 7, 9, 11, 13, 15, and 17, to determine if this level is lower in the active versus control groups (Appendix 6).

7.3.4 *In Vitro* Responses

Please refer to Section 9 for description of tolerance assays studies.

8 Adverse Event Collection and Reporting

Adverse events that are classified as serious, according to the definition of regulatory authorities, must be rapidly and adequately reported to the ITN/NIAID, the IND holder and regulatory authorities. This section provides definition of the types of adverse events and outlines a process for the appropriate reporting and follow-up procedures. Information in this section was obtained from the ICH guideline for Good Clinical Practice (mentioned previously), ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, and the Common Toxicity Criteria Manual Version 2.0 (June 1, 1999).

8.1 Definitions

8.1.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom (including an abnormal laboratory finding), or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Signs, symptoms, or diseases that are recorded as part of the participant's medical history, and then recur at the same intensity, are not adverse events. Thus, if "seasonal allergies" are captured in the history, any signs **(**including physical exam findings)or symptoms of this indication (such as sneezing; runny nose; itchy, watery eyes) will not be considered adverse events, unless they:

1. fulfill the definition of a serious adverse event or

2. have worsened and are clinically significant requiring medical intervention other than the use of the rescue medication, or

3. results in discontinuation of subject form the study

Throughout the study, the Investigator must record all adverse events on the appropriate adverse event form, regardless of the severity or relationship to study medication or procedure. The Investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

- Observation of the participant;
- Questioning the participant;
- Unsolicited complaint by the participant; or
- Discovery of abnormal clinical laboratory values or abnormal results of other evaluations (radiographs, ultrasound, ECG, etc.).

In questioning the participant the questioning should be conducted in an objective manner.

In the event of an abnormal laboratory value, the test should be repeated until it returns to normal or can be explained and the participant's safety is not at risk. Clinically significant laboratory abnormalities as determined by the Investigator must be recorded as adverse events, as well as recorded on the appropriate laboratory evaluation form(s).

8.1.2 Serious Adverse Event

A serious adverse event (SAE) or reaction is defined as any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect or precaution. This includes, but may not be limited to any of the following events:

- Death: A death occurring during the study or which comes to the attention of the Investigator during the protocol-defined follow-up after the completion of therapy, whether or not considered treatment-related, must be reported.
- Life-threatening: Any adverse therapy experience that places the participant or participant, in the view of the Investigator, at immediate risk of death from the reaction as it occurred.
- Hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity.
- Congenital anomaly/birth defect.
- Other conditions as specified in the protocol.
- An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical

judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

- For the purposes of this study, a serious adverse event will be reported in study participants who, because of a reaction during RIT and/or IT, receive epinephrine and, prior to receiving epinephrine, have at least one of the following: symptoms indicative of reduced cardiovascular and/or respiratory function, 15 mm or greater reduction in the mean blood pressure; other clinically significant changes in blood pressure or pulse rate; 20% or greater reduction in pulmonary function such as peak expiratory flow rate or FEV1.

SAEs will be collected from the time informed consent is obtained, to 30 days after study completion or to 30 days after a participant prematurely withdraws from the study.

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above criteria.

8.1.3 Unexpected Adverse Event

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with applicable product information, such as safety information provided in the package insert, investigational plan, Investigator's brochure, protocol or informed consent document.

8.2 Grading of Adverse Events

8.2.1 Toxicity Grading of Adverse Events

Toxicity grades are assigned by the study site to indicate the severity of adverse events occurring in ITN study participants. The ITN has adopted the use of the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) for application in adverse event reporting. The purpose of using the NCI-CTC system is to provide a standard language to describe toxicities, to facilitate tabulation and analysis of the data, and to facilitate the assessment of the clinical significance of all adverse events. The NCI-CTC provides the following grades and descriptions in the NCI-CTC Manual (Version 2.0). Adverse events should be recorded and graded 1 to 5 according to the NCI-CTC grades provided below:

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

Note: In contrast to the CTC guidelines, provided the NCI-CTC Manual (Version 2.0), all adverse events are to be reported and graded, whether or not related to disease progression or treatment.

8.2.2 Relationship to Study Therapy

The relationship or attribution between an adverse event and an investigational product is determined by the site Investigator or sub-Investigator and recorded on the appropriate Case Report Form and/or SAE Reporting Form. The NCI-CTC provides the following descriptors and definitions (1 category classified as unrelated [Code 1] and 4 categories classified as related [Codes 2-5]) for assigning an attribution to each adverse event as described below (Table 2).

Table 2 Attribution of Adverse Events

Code	Descriptor	Definition
"Unrelated" Category Code (1 Category):		
1	Unrelated	The adverse event is clearly not related to the investigational agent(s)
"Related" Category Codes (4 Categories):		
2	Unlikely	The adverse event is doubtfully related to the investigational agent(s)
3	Possible	The adverse event may be related to the investigational agent(s)
4	Probable	The adverse event is likely related to the investigational agent(s)
5	Definite	The adverse event is clearly related to the investigational agent(s)

The Investigator's determination of drug-relatedness (attribution) for each adverse event should be recorded in the source documentation.

For additional information, consult the Common Toxicity Criteria Manual and the Common Toxicity Criteria Document at the following URL: http://ctep.cancer.gov/reporting/ctc.html.

8.3 Serious Adverse Event Reporting

The following process for reporting a serious adverse event will ensure appropriate reporting compliance with the ICH guidelines.

8.3.1 Serious Adverse Event Identification and Determination of Reporting Timeline

When an Investigator identifies a serious adverse event (as defined above), the Investigator must notify the CRO Safety Reporting Center of the serious adverse event within 24 hours of discovery. The CRO Safety Reporting Center is contacted via the CRO 24-Hour SAE Reporting Hotline (1-800-201-8725). In addition to telephone reporting, these events must be entered on the Serious Adverse Event Form and the Adverse Event CRF. The Serious Adverse Event form and Adverse Event CRF should be faxed to the CRO Safety Reporting Center within 24 hours.

The Safety Serious Adverse Event Coordinator (SAEC) or Medical Liaison at the CRO Safety Reporting Center will notify the following individuals of the SAE: NIAID Project Manager/SMT Associate Leader, the ITN SMT Leader, and the ITN Medical Monitor. The NIAID Project Manager/SMT Associate Leader will notify the Primary Investigators. The CRO Safety SAEC or Medical Liaison and the ITN Medical Monitor will review the serious adverse event. If it is apparent that the event requires expedited reporting or discussion is required to determine this, the ITN Executive Clinical Director and/or ITN Associate Director Clinical Trials, NIAID Regulatory Officer, and NIAID Medical Officer will be included as well. If additional information is needed, the Investigator will be contacted.

The CRO Safety SAEC/Medical Liaison and ITN Medical Monitor (with input from the ITN Executive Clinical Director and/or ITN Associate Director Clinical Trials, NIAID Regulatory Officer, and NIAID Medical Officer as needed) will determine whether or not the serious adverse event reported by the site requires expedited reporting to the FDA by evaluating the following: (a) seriousness, (b) expected or unexpected, (c) related or not to the study drug.

Three possible reporting scenarios (to the appropriate health authorities) could arise after assessment of the event:

- No requirement to report. This would occur if the adverse event is deemed not serious by the CRO Safety SAEC/Medical Liaison and ITN Medical Monitor.
- Standard reporting is required. This would occur if the adverse event were classified as one of the following: (a) serious, expected and drug-related; (b) serious, expected and not drug-related; or (c) serious, unexpected and not drug-related.
- Expedited reporting is required. This would occur if the adverse event is considered serious, unexpected and drug-related. These events must be reported to the appropriate health authorities within 15 days unless the event is fatal or life-threatening, the latter must be reported within 7 days.

All PIs must report the serious adverse event to their respective Institutional Review Board (IRB) as mandated by their IRB.

8.3.2 Reporting Serious Adverse Events to Health Authorities

8.3.2.1 Standard Reporting

If the serious adverse event is classified such that standard reporting is appropriate, the CRO Safety Reporting Center summarizes the information within 1 working day and sends the summary to the SMT Leader, the NIAID Project Manager/Associate SMT Leader, and the ITN Medical Monitor. All PIs must report the serious adverse event to their respective IRBs. The IND holder must then include information regarding the serious adverse event in the IND annual report.

8.3.2.2 Expedited Reporting

If the serious adverse event requires expedited reporting as determined by the ITN Medical Monitor, ITN Executive Clinical Director, ITN Associate Director Clinical Trials, NIAID Regulatory Officer, and NIAID Medical Officer in collaboration with the Medical Liaison/Safety SAEC at CRO, the CRO Safety Reporting Center must immediately notify all of the above individuals as well as the SMT Leader, the NIAID Project Manager/Associate SMT Leader. The NIAID Project Manager/Associate SMT Leader will immediately notify the PI. The CRO Safety Reporting Center will then prepare the Medwatch Form as well as CIOMS-I (if international sites are conducting trials with the specific study drug) and an "Analysis of Similar Events" report (if requested by ITN) within two working days. These documents will be sent to the PI, NIAID Medical Officer, ITN Medical Monitor, ITN Executive Clinical Director, ITN Associate Director Clinical Trials, and NIAID Regulatory Officer for review and approval.

The NIAID Regulatory Officer will prepare a cover notification letter for the expedited report. The NIAID Regulatory Officer will also send a copy of the final expedited reporting package to the CRO Safety Reporting Center.

The final expedited reporting package will be sent by the NIAID Regulatory Officer, via overnight courier, to all PIs participating in trials with the specific drug, the IND holder, the SMT Leader, the NIAID Project Manager/SMT Associate Leader, ITN Medical Monitor, NIAID Medical Officer, identified members of the CTG and TAG (if appropriate), the study site monitors, and the supplier of the investigational drug and/or the holder of the Investigator's brochure. For expedited SAEs, all sites must place the notification letter and Medwatch/CIOMS with the Investigator's brochure.

8.4 Pregnancy (SAE Reporting Requirements)

Any pregnancy that occurs during a clinical study with an investigational drug must be reported as an SAE **for tracking purposes only**. All pregnancies that are identified during this study need to be followed to conclusion and the outcome reported. Female participants

should immediately inform the Investigator of any pregnancies and should be instructed by the Investigator to stop taking study medication. The Investigator should report all pregnancies within 24 hours (as described above in SAE Reporting) using the SAE form. The Investigator should counsel the participants and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participants should continue until conclusion of the pregnancy, and a follow-up SAE Reporting Form should be submitted detailing the outcome.

8.5 Updating Source Documentation

Documents describing the safety profile of a drug, such as the Investigator's brochure, should be amended, as needed, to ensure that the description of safety information adequately reflects any new clinical findings. Until these documents are updated, expedited reporting is required for additional occurrences of the reaction.

9 Tolerance Assay Studies

9.1 IgE Level

Omalizumab should cause a rapid decline in free IgE levels and an increase in total IgE.[30] Although omalizumab has not been shown to inhibit IgE synthesis in humans, the combination of omalizumab + IT may synergize to do so since IT decreases antigen-specific IgE production.[46] To test this hypothesis, mRNA will be prepared from B-cells stimulated with ragweed antigen and will be analyzed by real time-PCR for εmRNA. The following will also be measured: serum total and free IgE: antigen-specific IgE, IgG_1, IgG_4 and IgA. Omalizumab treatment should decrease ragweed specific IgE to undetectable levels until approximately 6 weeks after treatment, so these levels will not be monitored during omalizumab treatment periods.

9.2 Flow Cytometry – Surface Staining

Omalizumab pre-treatment should favor a shift from Th2 to Th1 paradigm via multiple mechanisms including: a shift in DC2 to DC1predominence; decreased FcεR1 expression leading to allergen presentation not favoring Th2 responses; and inhibition of proliferation of allergen-specific T-cells by inhibiting the binding of allergen-IgE complexes to CD23 on B lymphocytes. Allergen IT has been shown to favor the shift from Th2 to Th1 responses via a variety of mechanisms. Thus, the combination should be more efficient in inducing this important immunotolerogenic effect. The change in T-cell subsets consistent with these hypotheses will be determined by 4 color flow cytometry analyses. Baseline data will be obtained prior to treatment periods and compared for each participant with data acquired at various time points at each treatment, including during ragweed season and after discontinuation of therapy.

For example, human DC1 cells have been found to induce Th1 differentiation, whereas DC2 cells induce Th2 differentiation.[47] By reducing serum IgE levels, eosinophilia, and histamine release, omalizumab should facilitate a shift from a predominate DC2 paradigm to a DC1 paradigm, thus favoring a reduction in Th2 responses and an increase in Th1 responses to ragweed. Thus, DC1 and DC2 cells in peripheral blood, characterized by CD11c+ CD123- markers and CD11c-CD123 + markers, respectively, can be monitored by flow cytometry.

Additionally, a recent mouse study has demonstrated that regulatory CD4+CD25+ T-cells (IL-4 negative) up-regulated Th2 cell-mediated, antigen-induced eosinophil recruitment into airways of sensitized and challenged mice by modulating T helper cell differentiation to a Th2 type.[48] In contrast, in a mouse model of inflammatory bowel disease, which is induced by Th2 CD4+CD45RB^{hi} cells, mice were protected from disease by transfer of a CD4+ CD45RB^{lo}CD25+ population.[49] The regulatory cell effector mechanism is not known, although cell-cell contact and secretion of IL-10 or TGF-β have been implicated. [48;50] Recently, a number of investigators have isolated and characterized a similar population of regulatory T-cells from humans. [51] Although functional assays are not proposed at this time, we will determine if RIT or omalizumab + RIT lead to an alteration in the numbers of putative CD4+CD25+ regulatory T-cells. Specifically this population can be monitored by the presence or increase in numbers of CD4+CD25+CD45RB^{low} cells. Alternatively, the chemokine receptor CCR8, that is reported to be highly specific for this population, can be used as a marker for regulatory T-cells. [52]

9.3 Human Basophil Histamine Release

Omalizumab has previously been shown to decrease antigen-induced human basophil release in conjunction with decreases in both serum free IgE levels and FcεR1 expression on basophils.[27] To confirm these previous findings we will isolate and analyze blood basophils for stimulated histamine release due to ragweed and anti-IgE (anaphylactogenic anti-IgE antibodies) using previously published techniques. [53-55] Mixed leukocytes will be collected for this testing. Human basophil histamine release assays will only be done on a subset of participants.

9.4 Frozen PBMC- ELISPOT

Assessments of secreted cytokines in response to whole ragweed antigen stimulation will be performed on frozen PBMC's using the ELISPOT technique. Cytokines to be profiled include IL-4, IL-10, IL-5, IL-13, and IFN-gamma. These cytokine profiles will be indicative of an induced TH2 to TH1 shift in T-cell responses as well as induction of regulatory cytokines consistent with induction of tolerance. In order to determine whether the cellular source of secreted cytokines are CD8+ or CD4+ cells, blocking antibodies to CD4, CD8 or both will be utilized. For global assessment of cytokine release in response to whole ragweed antigen, supernatants from PBMC's in the ELISPOT cultures can be harvested and analyzed by the cytokine bead assay (CBA, Becton-Dickinson).

9.5 Apoptosis (TUNEL)

In addition to mechanisms cited above, a decrease in Th2 cell phenotype might occur through antigen-specific Th2 cell apoptosis. We will investigate whether preferential antigen-specific apoptosis of Th2 lymphocytes (CD4+/IL4+) is induced with RIT+/- omalizumab as demonstrated in a preliminary study with traditional IT. [56] Following antigen stimulation of PBMC, we will use a 3-color flow cytometric TUNEL assay to detect apoptosis-associated DNA strand breaks to indicate antigen-specific (IL-4+/CD4+) Th2 cell preferential apoptosis. We will also perform 6-day ^{3}H thymidine incorporation proliferation assays on PBMC with whole ragweed antigen and controls to determine if there is a decrease in antigen-specific T-cell proliferation.

9.6 Peripheral Blood Gene Expression Profiling – Gene CHIP/Real Time PCR

To further elucidate possible changes in cytokine and cellular profiles, gene expression profiling analyses will be performed on RNA isolated from peripheral blood. In addition, RNA will be isolated from PBMC, isolated from participant's pre- and post-therapy and subsequently cultured in the presence of whole ragweed antigen or PMA/Ionomycin. This system is necessitated by the need for an extremely sensitive assay system to detect allergen specific cytokine production. Real time PCR will allow for determination of known genes associated with TH1 and TH2 phenotypes as well as the discovery of new genes involved in this shift or tolerance induction. Examples of genes of interest include: IL-4, IL-5, IL-13, IL-10, IL-9, TGF-β, IFN-γ, MIP-1α, MIP-1β, and TARC.

9.7 DNA-HLA-SNP Genotype

DNA will be isolated from peripheral blood of trial participants and subjected to sequence based Class II typing and for future genotyping for potential SNP's associated with persons susceptible to allergic responses.

10 Statistical Considerations and Analytical Plan

10.1 Primary Endpoints

The primary endpoint will be the average daily allergy severity score, which will be calculated from participants' 5 symptom scores (sneezing; rhinorrhea/runny nose; itchy nose, throat, and palate; itchy, watery eyes; and nasal congestion/stuffiness) during the 2003 ragweed pollen season. Symptom scores are recorded twice daily (AM and PM). The ragweed pollen season begins when the ragweed pollen counts rise to 10 ragweed pollen grains/m^3/24 hours or above on two recorded consecutive days, and the ragweed pollen season ends when the ragweed pollen counts fall below 10 ragweed pollen grains/m^3/24

hours on two consecutive recorded days. The ragweed pollen season is from approximately August 15, 2003 to October 1, 2003, but varies among the sites. The sum of the individual symptom scores will be averaged over AM and PM to give a daily score. Each daily score will then be averaged to obtain one measure of the average daily allergy severity score for each participant.

10.2 Secondary and Tertiary Endpoints

The secondary and tertiary endpoints are outlined in 3.2.2 and 3.2.3

10.3 Sample Size and Statistical Power

A sample size of 168 participants was calculated using variation estimates from the placebo group of a previous study that investigated omalizumab for ragweed-induced SAR and had a similar design and primary efficacy variable of daily allergy symptom score.[15] The estimate of variation, then, is based on these severity scores. It is anticipated that anti-IgE therapy alone will not be as potent in inducing changes in the primary variable since omalizumab and IT involve multiple immunologic mechanisms of action. Assuming the combination of omalizumab and IT is better than IT alone with a mean difference of 0.40 and a standard deviation of 0.6, a significant difference (one-sided $p < 0.05$) would be detected with a power of 80% at $n = 29$. Therefore, the primary analysis will be a 1-sided test with an alpha level of 0.05. Assuming an approximately 30% dropout rate, 42 participants in each treatment arm should allow sufficient power to detect significant differences. It is anticipated that up to 300 potential participants will be screened in order to randomize 168 eligible participants into the Study Treatment arms.

10.4 Randomization, Stratification and Blinding

The participants will be randomized to one of the 4 treatment groups at 1:1:1:1 at the beginning but presented to the pharmacist or designee as 2 randomization episodes. In the first episode, participants are randomized to either omalizumab or its placebo. At the completion of the first episode, the pharmacist or designee will be presented the randomization codes for the second episode, either IT or its placebo.

A centralized randomization procedure will be used by the study statistician to generate the randomization schedule with site and participant ID numbers. The site-specific randomization schedule will be sent via Fed-Ex to the appropriate site pharmacist or designee. Individual medication assignment for this blinded study will be provided in sealed disclosure envelopes. To obtain a treatment assignment for an individual study participant eligible for the study, the coordinator at the local site will complete the appropriate Enroll New Participant form and send to the site pharmacist or designee to receive the treatment assignment and participant numbering information. The site pharmacist or designee will provide the assignment information in a sequential fashion according to the site-specific randomization schedule. No one at the local site, other than the designated individual(s) at

the local pharmacy, will have knowledge of the treatment assignment for an individual participant, unless required for managing the participant's care.

10.4.1 Unblinding Authorization

Emergency unblinding of a patient for safety purposes is to be handled through the Investigators, Medical Monitor and the Site Pharmacist or designee. In the case of a life-threatening event, the Medical Monitor of the trial should be contacted prior to unblinding if at all possible. If the Medical Monitor cannot be reached, the Investigator will make the decision. For all other events that require unblinding, the Medical Monitor must be contacted first. The Investigator in consultation with the Medical Monitor, will discuss the unblinding of any individual participant. If the decision is to unblind, the Investigator will inform the Principal Investigator and the Medical Monitor will inform the SMT members of the unblinding event. The Medical Officer at NIAID will be responsible for notifying the DSMB.

Any unblinding event will require a full written account of the event(s) (including the name of Medical Monitor who was notified, date, time, and reason for unblinding) necessitating the disclosure (unblinding) of study medication for an individual patient. During site visits, the Site Monitor should check that the disclosure envelopes are intact and in a secure yet accessible location for study personnel. All disclosure envelopes must be returned to the Site Monitor at the end of the study. If any of the disclosure envelopes are opened, the Site Monitor must verify that the ITN Medical Monitor was notified and a written account (described above) was completed. The reasons for unblinding each individual's Study Treatment should be included in the final study report.

If an entire study needs to be unblinded (due to an approved interim analysis, final analysis, or study termination as described in Section 3.7) this will require ITN/NIAID approval. This approval will be obtained through sign off of a study unblinding authorization form. Randomization lists will be maintained and secured at PPD.

10.5 Statistical Analyses

10.5.1 Analysis Populations

The following analysis populations will be applied for efficacy and safety analyses:

- ITT population: All participants randomized will be included in the intent-to-treat (ITT) population. This will be the primary study analysis.
- PP population: All participants completing study therapy per protocol will be included in the per-protocol (PP) population. By study design, the PP population will be used for the tertiary analyses due to the extended follow-up phase of the study including only those participants completing study therapy per protocol.

Safety population: All participants who receive any study therapy will be included in the safety population.

10.5.2 Description of Baseline Characteristics and Demographics

Summary descriptive statistics for demographic and baseline characteristics will be provided by treatment group and overall, for all enrolled participants. Demographic characteristics will include age, race, gender, body weight (kg), and height (cm).

10.5.3 Medical and Surgical History

Medical and surgical history will be collected including the existence of current signs and symptoms and clinical significance for each body system.

10.5.4 Use of Medications

All medication use will be coded using the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

10.5.5 Study Completion

The number and percentage of participants who fail to complete the study, are lost to follow-up, and the reasons for discontinuation (e.g., adverse events) will be presented for each treatment group, by site, and overall.

10.5.6 Efficacy

Variables will be summarized and tabulated separately for each of the treatment groups. The data analyses will be based on the intent-to-treat population. The primary analysis will compare the combination treatment of omalizumab + IT versus IT alone. Other comparisons

of the four treatment arms will be included in the secondary analyses. The tertiary endpoints over the 2004 ragweed season will compare each treatment group versus the placebo treatment group. Thus, the tertiary analyses will test persistent treatment effects in each active treatment group compared to the placebo treatment group (no active treatment).

The between treatment analysis of symptom severity scores, medication use, and RQLQ scores will be performed using an analysis of variance model (ANOVA) for multivariate analysis and two-sample t-test for univariate analysis. Baseline characteristics as well as the primary and secondary efficacy variables will be tabulated and compared using ANOVA methods accounting for within participant correlation. The treatment by site interaction, if any, will be assessed. When indicated, a Cochran-Mantel-Haenszel test controlling for study site will be used to inferentially compare the treatment groups with respect to categorical variables. Missing values in the multivariate analyses will be imputed when appropriate. When calculating the averages of daily scores, the average will be based on the non-missing observations.

Descriptive statistics (number, mean, standard deviation, median, minimum, maximum) will be presented for all continuous and ordinal variables. Frequencies and percentages will be calculated for all categorical variables. Descriptive statistics will be performed for the morning total allergy symptom score, the evening total allergy symptom score, the daily mean of the morning and the evening total allergy symptom score, and the individual symptom scores for the five components of the total allergy symptom scores.

The RQLQ questionnaire is a 28-item questionnaire assessing an overall score in 7 domains of rhinitis-related quality of life (3 for sleep, 7 for non-nose/eye symptoms, 4 for eye symptoms, 3 for practical problems, 4 for nasal symptoms, 3 for activities and 4 for emotional). [15;57] A 7-point scale will be used for rating questions. For each participant, results will be expressed as the mean score for each domain as well as for the overall QOL.

These results will be summarized descriptively by treatment group and visit. Treatment group comparisons will be made using ANOVA models adjusting for site.

It is assumed that participants treated with omalizumab in combination with IT will have better average daily allergy severity scores than participants on IT alone. Hence, a one-sided significance test will be used for the primary efficacy analysis. The primary outcome will be considered significant if one-sided $p < 0.05$. All other efficacy outcome measures will be considered statistically significant if two-sided $p < 0.05$.

Tertiary analyses will use data from the 2004 ragweed season and will be comparable to the analyses performed on the 2003 ragweed season data where applicable. The analysis of the change in average allergy severity scores between the 2003 and 2004 ragweed seasons will use a noninferiority test to determine if the difference between omalizumab + ragweed IT and placebo during the 2004 ragweed season is equivalent to the same difference during the 2003 ragweed season.

P-values will be presented with rounding to three decimal places. If a p-value is less then

0.001, it will be reported as "<0.001". If a p-value is greater then 0.999, it will be reported as ">0.999".

SAS Version 6.12 or later (SAS Institute, Cary, NC) will be used for statistical analyses.

10.5.7 Safety

Safety evaluation will be performed for the Safety population. Safety parameters to be displayed for each treatment group will include adverse events (AE) as well as changes in vital signs, and changes in routine laboratory values between baseline and study completion.

Adverse events will be classified using the NCI-toxicity scale and coded using the MedDRA dictionary. The number of events and the incidence of adverse events by body system and preferred term will be summarized. Adverse events by maximum severity and relationship to study therapy will also be assessed by treatment groups. Separate summaries will be provided for all treatment emergent AE's, serious AE's, treatment-related AE's and AE's leading to study discontinuation. Adverse events occurring before study entry, but not worsening or not related to study therapy during study will not be considered as treatment emergent AE's. Descriptive statistics for changes from baseline summary will be provided for laboratory and vital sign results.

10.6 Planned Interim Analyses

Adverse event data will be monitored and analyzed throughout the study by the independent Data and Safety Monitoring Board (DSMB). Interim analyses are planned for efficacy and safety data upon completion of study visits 17 and 20, by all enrolled study participants, and upon completion of all CRFs and resolution of all discrepancies.

10.7 Procedures for Reporting Deviations from the Original Statistical Plan

The principal features of the design of this study and of the plan for statistical analysis of the data are outlined in this protocol and subsequent statistical analysis plan (SAP). Any changes in these principal features will require a protocol or SAP amendment, which would then be subject to review by the independent Data and Safety Monitoring Board (DSMB), study sponsor(s), and regulatory agencies. These changes will be described in the final report as appropriate.

11 Access to Source Data / Documents

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants and donors participating in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality

assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational site will normally be notified in advance of auditing visits.

12 Quality Control and Quality Assurance

The Investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The Investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency and accuracy of all documented data.

To ensure the reliability of the data recorded in the database, double data entry will be used for all fields on the CRF. The data will be verified by a series of computerized edit checks, and all relevant data queries will be resolved on an ongoing basis. When the CRFs are complete, they will be reviewed and signed by the Investigator and returned to the sponsor or the contract research organization (CRO). All data from the original signed CRF will be entered in the database, and a comparison program will be run again. All discrepancies will be reviewed, and any resulting queries will be resolved with the Investigator and amended on the database. All elements of data entry (i.e. time, date, verbatim text and the person performing the data entry) are recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

13 Ethical Considerations and Compliance with Good Clinical Practice

13.1 Statement of Compliance

This trial will be conducted in compliance with the protocol, current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics review committee or Institutional Review Board

(IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

13.2 Informed Consent

The informed consent form is a means of providing information regarding the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, taking Study Treatment and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect a participants' participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The Investigator, in the presence of a witness, will review the consent and answer questions. The participant will be informed that their participation is voluntary and they may withdraw from the study at any time, for any reason.

All study participants considered for enrollment in the extended follow-up phase will be asked to review and sign an addendum to the original informed consent. The addendum will clearly outline any additional procedures the participant must undergo. The extended follow-up does not involve any study treatment. A copy of the addendum will be given to the participant for review, along with the previously signed informed consent. The Investigator or designee, in the presence of a witness, will review the addendum and answer any questions. The participant will again be informed that their participation is voluntary, that they are not required to enroll in the extended follow-up, and that they may withdraw from the study at any time, for any reason.

13.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number, and these numbers rather than names will be used to collect, store and report participant information.

14 Publication Policy

The ITN policy on publication of study results will apply to this study. Details regarding the policy statement may be found by authorized participants on the ITN internet website at http://www.immunetolerance.org.

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16 Appendix 1: Screening Procedures

1. The medical history, ragweed pollen history and medication history will be obtained including any previous ragweed immunotherapy and omalizumab administration.

2. A complete physical examination performed by an Investigator will include the following:

    - General appearance, hair and skin, lymphatics, head, eyes, nose, throat, neck, lungs, cardiovascular, abdomen, musculoskeletal, neurological and mental status.

3. Vital signs will include:

    - Temperature, respiratory rate, blood pressure, pulse and height/weight.

Other than at the screening visit, the height will be deleted for the remainder of the study.

4. Electrocardiogram will include a standard 12-lead ECG reviewed by an Investigator.

5. Allergy skin testing will include:

    - Prick tests placed approximately 5 cm apart using a separate needle for each test;
    - Positive control (histamine base 1 mg per mL);
    - Negative control (extract diluent); and
    - Ragweed extract at 1:20 weight per volume.

The skin tests results will be graded 15-20 minutes after application.

The skin test reactions will be recorded by outlining the wheal and erythema with a fine tip black pen and placing transparent tape over the outlying skin reaction.

The tape will then be lifted along with the adherent ink off the skin and placed into the source documents.

The skin test measurement will be done as follows:

The longest and longest perpendicular diameters on both wheal and erythema will be recorded on the source documents as mean values.

A positive skin test will be one in which the response to ragweed extract will be at least 3 mm or greater than the diluent control.

6. Hematology will include:

    - Hemoglobulin, MCV, white blood cells, red blood cells, platelet count, hematocrit, MCH, WBC differential and MCHC.

7. Blood chemistry will include:

    - BUN, creatinine, glucose, uric acid, total protein, albumin, triglycerides, cholesterol, CK, potassium, sodium, calcium, total bilirubin, alkaline phosphatase, ALT, AST, gamma GT and LDH.

8. A urinalysis will include:

    - Appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, blood and microscopic evaluation.

In females of child-bearing potential, a pregnancy test will also be done.

9. Total IgE will be measured.

10. Pulmonary function test (FEV_1, FVC and FEV_1/FVC)

17 Appendix 2: Omalizumab Dosing Schedule

Dosing

The following is the dosing table based on body weight and IgE levels for 4-week dosing intervals. The dose for each cell was calculated based on the maximum body weight and maximum baseline IgE for that cell to deliver a minimum of 0.016 mg/kg/IgE (IU/mL) q 4 week equivalent dose, therefore all others (below maximum body weight and serum IgE level) in the cells will receive doses exceeding the 0.016 mg/kg/IgE (IU/mL) equivalent.

	Total milligrams of Omalizumab required per 4-week interval						
	Body weight (kg)						
Baseline IgE (IU/mL)	>30–40	>40–50	>50–60	>60–70	>70–80	>80–90	>90–120
>10–100	150	150	150	150	150	150	300
>100–200	150	300	300	300	300	300	450
>200–300	300	300	300	450	450	450	600
>300–400	300	450	450	450	600	600	
>400–500	450	450	600	600	750	750	
>500–600	450	600	600	750			
>600–700	450	600	750				

9. Doses > 300 mg every 4-week interval are split into 2 equal doses administered every 2 weeks (shaded cells).
10. Doses ≤ 300 mg per 4-week interval are administered once every 4 weeks.
11. The maximum dose at any given visit for patient would be 375 mg (~19 mg/kg for patients with the smallest body weight).
12. Dose volumes are ~1.2 mL for each 150-mg unit dose based on the Phase III formulation of omalizumab at a concentration of 125 mg/mL.
13. No injection at a single site is to exceed 1.2 mL (150 mg).
14. Doses requiring greater than a single 1.2 mL injection are to use multiple injection sites.
15. Doses greater than 300 mg (2 × 1.2 mL injections) are to be administered every 2 weeks.
16. Patients requiring doses that are not integer multiples of 150 mg (i.e., 225 mg and 375 mg) are to receive multiple injections with total volumes of 1.8 mL (1.2 and 0.6 mL injections for 225 mg) or 3.0 mL (1.2, 1.2, and 0.6 mL injections for 375 mg).

.

Reconstitution Instructions

Reconstituted omalizumab is stable for up to 24 hours at 2°–8°C and for 8 hours at controlled room temperature (15°–30°C).

The lyophilized product typically takes 10–20 minutes to dissolve completely; some vials may require more than 20 minutes. The fully reconstituted product will appear clear or slightly opaque and may have a few small bubbles or foam around the edge of the vial. Because the reconstituted product is somewhat viscous, care must be taken to withdraw the entire product from the vial before expelling any air or excess solution from the syringe in order to obtain the full 1.2-mL dose.

The reconstitution steps are as follows:

1. Carefully draw 1.3 mL of SWI into a 3 cc syringe equipped with a 1-inch, 18-gauge needle.
2. With the vial placed upright on a flat surface, insert the needle and transfer the SWI into the vial using standard aseptic techniques. Inject the SWI directly onto the product. DO NOT remove the needle and syringe from the upright vial.
3. Keeping the vial in an upright position, vigorously swirl the upright vial (do not shake) for approximately 90 seconds to evenly wet the powder. Remove the syringe and needle.

   Note: The lyophilized product typically takes 10–20 minutes to dissolve completely.
4. To aid in dissolution after completing Step 3, gently swirl the upright vial for 5–10 seconds approximately every 5 minutes in order to dissolve any remaining solids.
5. When the product is fully dissolved, there should be no visible gel–like particles in the solution. It is safe and acceptable to have small bubbles or foam around the edge of the vial. The reconstituted product will appear clear or slightly opaque.

   Note: Some vials may take longer than 20 minutes to dissolve completely. If this is the case, repeat Step 4 until there are no visible gel–like particles in the solution.
6. Invert the vial for 15 seconds in order to allow the solution to drain toward the stopper. Using a 3 cc syringe equipped with a 1-inch, 18-gauge needle, insert the needle into the inverted vial. Position the needle tip at the very bottom of the solution in the vial stopper when drawing the solution into the syringe. Before removing the needle from the vial, pull the plunger all the way back to the end of the syringe barrel in order to remove all of the solution from the inverted vial.
7. Replace the 18-gauge needle with a 25-gauge needle for SQ injection.
8. Expel air, large bubbles, and any excess solution in order to obtain the required 1.2-mL dose. A thin layer of small bubbles may remain at the top of the solution in the syringe. Because the solution is slightly viscous, the injection may take 5-10 seconds to administer.

9. The vial delivers 1.2 mL (nominal 150 mg) of omalizumab. For a 75 mg dose, draw up 0.6 mL into the syringe and discard the remaining product.

Omalizumab is for single-use only and contains no antibacterial preservatives. The solution may be used for SC administration within 8 hours following reconstitution when stored in the vial at 2°C–8°C (36°–46°F), or within 4 hours of reconstitution when stored at room temperature.

Storage Requirements

Vials of lyophilized omalizumab and placebo must be shipped at ambient temperature (priority overnight) and must be placed in a 2°–8°C (36°–46°F) refrigerator immediately upon receipt to ensure optimal retention of physical and biochemical integrity. The freeze-dried cakes are white to off-white, and after reconstitution with SWI, USP, should appear as a colorless to pale yellow opalescent solution. Omalizumab may be sensitive to shear-induced stress, such as agitation or rapid expulsion from a syringe. Vigorous handling of solutions of omalizumab may result in aggregation of the protein and cloudy solutions. Expiration dating is indicated on the vial label.

18 Appendix 3: Immunotherapy Protocols and Short Ragweed Extract

Short Ragweed Extract for Screening Skin Prick Test: Glycerinated Short Ragweed Extract was provided to the sites for skin prick tests performed only on the first screening visit, to determine allergy to ragweed. 267 patients were screened with this product. The manufacturer's details are as follows:

ALK-Abello, Inc.
1700 Royston Lane
Round Rock, TX 78664
Tel: (512) 251-0037
Fax: (512) 251-5882

Aqueous Short Ragweed Extract for Skin Reaction Tests (Intradermal Skin Tests), and Nasal Allergen Challenge Tests: During the initial screening phase, the study sites also received aqueous short ragweed extract from ALK-Abello to perform the nasal allergen challenge tests and intradermal skin tests on the second screening visit. The aqueous ragweed extract used for these baseline tests had a concentration of 561 µg/mL. 177 patients received the aqueous short ragweed from ALK-Abello. In order to prepare the ragweed for the skin tests and nasal challenges the pharmacist or the designee prepared a 50% dilution of the ragweed extract. The 50% dilution contained approximately 280 µg/mL of *Amb a 1*.

After June 6, 2003 Greer Laboratories, Inc. provided the aqueous short ragweed extract for the remainder of this study. Two concentrations of the aqueous ragweed were provided, 400 µg/mL of *Amb a 1* for the rush immunotherapy injections and the immunotherapy injections, and 546 µg/mL of *Amb a 1* for the remaining skin tests and nasal challenges that will be performed through follow-up visits. For the 2004 extended follow up phase of the protocol, Greer Laboratories, Inc. provided the aqueous short ragweed extract at 368 µg/mL of *Amb a 1* for the nasal challenges and skin testing. In order to prepare the ragweed for the skin tests and nasal challenges the pharmacist or the designee is required to first prepare a dilution of the ragweed extract, which contains approximately 273 µg/mL *Amb a 1* (during the treatment and follow up phase) and 283 µg/mL *Amb a 1* (during the 2004 extended follow up phase). This diluted solution is considered the "1:10" vial for skin testing and nasal allergen challenge testing.

Short Ragweed Extract for RIT and IT Injections: As stated above, after June 6, 2003, the study sites received commercially available Short Ragweed Extract (400 of *Amb a 1*) in aqueous form, from Greer Laboratories, Inc. for rush immunotherapy and immunotherapy. This aqueous ragweed was packaged into patient specific study medication kits and distributed by EMINENT Services, Inc.

Procedure: Perform all dilutions using sterile 1 mL syringes and enter required information on respective vial labels.

1. Transfer 1 mL of Short Ragweed Extract 400 AgE units/mL (AgE = Antigen E Units which is equivalent to µg/mL) or Histamine Solution, 1.25 mg/mL in to each

of the three green vials (D1, D2, and D3) and mix the contents by inverting the vial several times. Use vial D1 for RIT and 1–4 weeks IT, D2 for 5–8 weeks IT, and D3 for 9–12 weeks IT.

2. Transfer 1 mL from green vial D1 into red vial (C) and mix the contents by inverting several times.
3. Transfer 1 mL from red vial C into blue vial (B) and mix the contents by inverting several times.
4. Transfer 1 mL from blue vial B into yellow vial (A) and mix the contents by inverting several times.

For kits containing ragweed extract only:

5. After completion of steps 1–4, remove 6 mL of solution using a 10-mL syringe from each of the red, blue, and yellow vials, and discard into the excess solution vial provided. Record the subject ID# on the vial label.

The manufacturers details for the aqueous short ragweed extract used after June 6, 2003 is as follows:

Greer Laboratories, Inc.
639 Nuway Circle, P.O. Box 800
Lenoir, NC 28645
Tel: (828) 754-5327/ (800) 378-3906
Fax: (800) 419-7302

Placebo Histamine Solution: Placebo Histamine solution will be manufactured by University of Tennessee at concentration of 1.25 mg/mL and packaged in 10-mL glass vials with 10-mL fill volume. The manufacturer's details are as follows:

Department of Pharmaceutical Sciences
University of Tennessee
26 South Dunlap Street
Memphis, TN 38163
Tel: (901) 448-4443 Fax: (901) 448-6092

Study Medication Kits: The sites received one Study Medication Kit per participant containing either the Short Ragweed Extract or Placebo Histamine Solution packaged in glass vials (10 mL/vial). The kit also contained 6 color coded vials with diluent to prepare further dilutions along with instructions. These medication kits covered both the RIT and IT phases. The Study Medication Kits were assembled and distributed by the following contractor:

EMINENT Services Corporation
7495 New Technology Way
Frederick, MD 21703-9401
Tel: (240) 629-1972 Fax: (240) 629-3298

The site pharmacist or designee is not blinded and will prepare the required dilutions per protocol using these vials. To perform each dilution 1 mL of the previous dilution is transferred in to the next dilution vial.

Study Medication Kit Contents

Short Ragweed Extract/Placebo Histamine Solution	1 vial
Diluent vials, D1-D3 (green 1st dilution), 9 mL/Vial	3 vials
Diluent vial, C (red 2nd dilution)*	1 vial (RIT Only)
Diluent vial, B (blue 3rd dilution)*	1 vial (RIT Only)
Diluent vial, A (yellow 4th dilution)*	1 vial (RIT Only)

*The volume of diluent in the vials (for second, third, and fourth dilutions) is 9 mL in kits with Short Ragweed Extract and 3 mL in kits with Placebo Histamine Solution.

Rush Immunotherapy

A maintenance solution, as described above, will be prepared that contains approximately 40 μg/mL of *Amb a 1.* Serial dilutions will be made to the maintenance solution as described in the table below.

Table 1. Rush Immunotherapy

Time (hr)	Volume (mL)	Active RIT (Ragweed Maintenance Dilution)*	Placebo RIT (Ragweed Control)
0	0.30	1:1000	0.002mg/mL histamine
0.5	0.10	1:100	0.008 mg/mL histamine
1.0	0.30	1:100	0.008 mg/mL histamine
1.5	0.05	1:10	0.032 mg/mL histamine
2.0	0.15	1:10	0.032 mg/mL histamine
3.0	0.30	1:10	0.032 mg/mL histamine
• The maintenance solution contains approximately 40 μg/mL *Amb a 1* (1:1) • The maintenance histamine concentration is 1.25 mg/ml • RIT must be completed at least 3 weeks prior to the start of ragweed season.			

- Times of injections will be the same across all treatment groups.
- Active RIT:
  Receives short ragweed extract as indicated.
- Placebo RIT:
  Receives same volume of placebo solution (to match ragweed group) containing diluent for ragweed plus histamine. This should cause local reaction and pruritus at the injection site in a similar fashion to ragweed enhancing the blinding process. During the RIT period, either a physician will be present in the room where the RIT is being administered or a nurse trained and experienced in anaphylaxis recognition and treatment will be present in the room and a physician immediately available with a dedicated open phone line and be within a 1-minute walk from the RIT room. All the

personnel involved in the RIT procedures have received proper training to administer RIT and to recognize and treat systemic reactions and possible anaphylactic shock.

- During the RIT, vital signs will be monitored every 30 minutes.
- Participants will be monitored for 2 hours following the last RIT injection.
- To increase the safety of rush immunotherapy, prior to each injection participants will have vital signs and peak expiratory flows measured and will be asked about any adverse symptoms which will be recorded. If participants have any symptoms indicating either a nonlife-threatening systemic reaction or anaphylactic shock, they will be treated according to published guidelines by the American Academy of Allergy, Asthma and Immunology [59] and ACLS guidelines for the treatment of anaphylactic shock. Since the prompt recognition of systemic reactions and the immediate use of epinephrine are the mainstays of therapy, all study personnel will be trained to recognize and treat acute allergic reactions with epinephrine and a physician will be in the vicinity at all times. All three sites will have access to Code Blue teams in the hospital setting at which the therapy will be administered. In addition, the following equipment and reagents will be available at each site according to the recommendations of the World Health Organization paper[39]:

  1. Stethoscope and sphygmomanometer;
  2. Tourniquets, syringes, hypodermic needles and large bore (14 gauge) needles;
  3. Aqueous epinephrine HCl 1:1000;
  4. Equipment for administering oxygen;
  5. Equipment for administering intravenous fluids;
  6. Oral airway;
  7. Antihistamine for injection;
  8. Corticosteroids for intravenous injection; and
  9. Vasopressor.

- Participants with systemic reactions (anaphylactic reactions) will be treated according to AAAAI guidelines[59] and current ACLS practices and stop receiving Study Treatment. Systemic reactions will be defined as any of the following symptoms: wheezing, flushing, urticaria, angioedema, or a drop in the mean blood pressure of 15 mm or greater. For participants who have other symptoms including light headedness, itching, abdominal pain or nausea, the discretion of the Investigator will be used to treat the participant and determine whether that participant will continue in the study.

- Participants must achieve a minimum of 0.15 mL of the 1:1 maintenance dilution (6 µg *Amb a 1*) by the third week of IT. If tolerated, ragweed immunotherapy will be increased to maximum dose of 0.3 mL. If large local reactions that are not tolerated by the participant are noted with doses greater than 0.15 mL, the investigator at his discretion may lower the next dose to the previously tolerated dose. Thus, the highest tolerated dose, either 0.15, 0.2, or 0.3 mL, will be administered. Once the dose is reduced, it cannot be increased. If participants cannot tolerate a minimum dose of 0.15 mL of 1:1 maintenance dilution, the Study Treatment must be discontinued.

- Large local reactions (i.e. $\geq$ 4 cm diameter) will not be considered a reason to modify the dosing regimen (i.e. giving the same dose a second time). Large local reactions will only lead to discontinuation of the participant if, in the view of the Investigator, there is considerable participant discomfort or the administration of increasing doses of immunotherapy could be compromised.

Table 2. Weekly Ragweed Immunotherapy Treatment* Schedule

Week	Volume, mL	*Amb a 1*, μg
1	0.05	2
2	0.10	4
3	0.15	6
4	0.20	8
5	0.30	12
6	0.30	12
7	0.30	12
8	0.30	12
9	0.30	12
10	0.30	12
11	0.30	12
12	0.30	12

*Treatment products are Short Ragweed Extract 1:1 maintenance dilution with 40 μg/mL *Amb a 1*, as described above, or Placebo Histamine Solution (0.125 mg/mL).

Systemic allergic reactions, as defined in this appendix, will be treated as detailed above and necessitate discontinuation of the participant from continued treatment in the protocol.

19 Appendix 4: Schedule of Assessments

ITN019AD Allergen Immunotherapy Co-Administered with Omalizumab-Treatment Phase																		
Test	**Screening**		**Omalizumab Pre-treatment***			**RIT**	**Omalizumab/Placebo* + Immunotherapy/Placebo**											
Visit[a]	**-01**	**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Week	**-12 to -10**	**-11 to -9**	**-9**	**-5**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
GENERAL ASSESSMENTS & PROCEDURES																		
Informed Consent	X																	
Medical History	X																	
Ragweed Pollen History	X																	
Medication History	X																	
Complete Physical Exam	X																	
Interim Medical History		X	X	X	X	X	X	X	X	X			X				X	X
Interim Physical Exam		X	X	X	X	X			X				X				X	
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Allergy Skin Tests	X																	
Allergy Skin Reactions Test [b]		X									X							
Nasal allergen challenge (including nasal scraping)		X																
Nasal Exhaled NO		X					X		X		X		X		X		X	
Nasal Scraping		X																
Pulmonary Function Test	X																	
12-lead ECG	X																	
Eligibility/Continued Eligibility	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization			X															
Dispense 180 mg fexofenadine					X													
Dispense rescue medication			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Rescue medication use, pill count				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Issue participant diary cards						X	X	X	X	X	X	X	X	X	X	X	X	X
Collect and review diary cards							X	X	X	X	X	X	X	X	X	X	X	X
Quality of Life Questionnaire			X				X			X			X			X		
Adverse Event Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

*Administration of >300 mg of omalizumab will be split into two equal doses, 2 weeks apart (Appendix 2). Those requiring 2-week injections (Q2W) will have omalizumab injections at Weeks -9, -7, -5, -3, -1, 1, 3, 5, 7, 9, and 11. Participants with Q2W dosing will have to return for interim visits (Weeks -7 and -3) when pregnancy, adverse events, and vital signs will be assessed. For these participants, additional pregnancy tests will be performed at Visits 5, 9, and 13.

[a] Visits 1-16 should occur within ± 3 days of the scheduled day; visits 17-20 should occur within $\pm$ 5 days of the scheduled day. An unscheduled visit (USV) will be necessary if the patient needs to repeat an RIT or an IT injection or to comply with any other aspect of the required study protocol. (see Section 6.6). [b] Participants will be examined 16-24 hours after the skin testing and nasal allergen challenge procedures.

(Continued from previous page)

ITN019AD Allergen Immunotherapy Co-Administered with Omalizumab-Treatment Phase																		
Test	**Screening**		**Omalizumab Pre-treatment***			**Rush IT**	**Omalizumab/Placebo* + Immunotherapy/Placebo**											
Visit[a]	**-01**	**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Week	**-12 to -10**	**-11 to -9**	**-9**	**-5**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
LABORATORY ASSESSMENTS																		
Total IgE	X																	
CBC with differential	X		X	X	X				X				X				X	
Blood Chemistries	X		X	X	X				X				X				X	
Triglycerides, cholesterol	X		X	X	X				X				X				X	
Gamma GT	X		X	X	X				X				X				X	
Urinalysis	X		X	X	X				X				X				X	
Urine Pregnancy Test*	X	X	X	X	X		*		X		*		X		*		X	
Serum for HAHA testing		X				X	X				X				X			
IMMUNOLOGIC STUDIES																		
Peripheral Blood Gene Expression Profile – Real Time PCR/Gene Chip		X				X	X				X				X			
Flow Cytometry – Surface Staining		X				X	X				X				X			
DNA-HLA-SNP		X																
Apoptosis (TUNEL)		X				X	X				X				X			
Frozen PBMC - ELISPOT		X				X	X				X				X			
Antigen Specific Antibodies		X				X	X				X				X			
Free IgE						X	X				X				X			
Basophil Histamine Release		X				X	X				X				X			
STUDY DRUG ADMINISTRATION[c]																		
Omalizumab/placebo *			X	X	X		*		X		*		X		*		X	
RIT/ placebo RIT						X												
Immunotherapy/placebo IT							X	X	X	X	X	X	X	X	X	X	X	X

*Administration of >300 mg of omalizumab will be split into two equal doses, 2 weeks apart (Appendix 2). Those requiring 2-week injections (Q2W) will have omalizumab injections at Weeks -9, -7, -5, -3, -1, 1, 3, 5, 7, 9, and 11. Participants with Q2W dosing will have to return for interim visits (Weeks -7 and -3) when pregnancy, adverse events, and vital signs will be assessed. For these participants, additional pregnancy tests will be performed at Visits 5, 9, and 13.

[a] Visits 1 through 16 should occur within $\pm$ 3 days of the scheduled day; visits 17-20 should occur within $\pm$ 5 days of the scheduled day.

[b] Participants will be examined 16-24 hours after the skin testing and nasal allergen challenge procedures.

[c] On days that both IT and omalizumab are administered, omalizumab will be injected 60 minutes after the IT.

Tests	**Follow-up (2003 ragweed season)**				**Extended Follow-up (2004 ragweed season)**							
Visit [a]	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Week	**13**	**19**	**31**	**43**	**55**	**57**	**59**	**61**	**63**	**65**	**67**	**71** [b]
Complete Physical Exam				X								
Interim Medical History	X		X	X	X	X	X	X	X	X	X	X
Interim Physical Exam	X		X		X							X
Vital Signs	X	X	X	X	X							X
Allergy Skin Reactions Test [c]	X	X	X	X	X							X
Nasal allergen challenge (nasal scraping)[c,d]	X	X	X	X	X							X
Nasal Exhaled NO	X											
Nasal Scraping	X	X	X	X								
12-lead ECG	X											
Confirmation of Eligibility	X	X	X	X	X	X	X	X	X	X	X	X
Addendum to the Informed Consent				X[e]								
Dispense rescue medication	X	X	X	X[f]	X	X	X	X	X	X	X	
Rescue medication use, pill count	X	X	X	X	X	X	X	X	X	X	X	X
Issue participant's diary cards					X	X	X	X	X	X		
Collect and review diary cards	X					X	X	X	X	X	X	
Quality of Life Questionnaire	X						X		X		X	
Adverse Event Assessment	X	X	X	X	X	X	X	X	X	X	X	X
LABORATORY ASSESSMENTS												
CBC with differential	X			X								
Blood Chemistries	X			X								
Triglycerides, cholesterol	X			X								
Gamma GT	X			X								
Urinalysis	X			X								
Urine Pregnancy Test	X		X	X			X					X

[a] Visit 17 must occur at least 1 week (but no later than 4 weeks) after the end of 2003 ragweed season. Visits 1 through 16 should occur within ± 3 days of the scheduled day; visits 17-28 should occur within ± 5 days of the scheduled day.
[b] Visit 28 must occur at least 2 weeks post-ragweed season in 2004.
[c] Participants will be examined 16-24 hours after the skin testing and nasal allergen challenge procedures.
[d] At visits 21 and 28, nasal challenge will be performed only in a select group of patients.
[e] The addendum to the informed consent should be signed at Visit 20 or at the soonest convenience thereafter.
[f] Rescue medication for Visit 20 may only be dispensed after the participant has signed the addendum to the informed consent.

Tests	**Follow-up (2003 ragweed season)**				**Extended Follow-up (2004 ragweed season)**							
Visit [a]	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Week	**13**	**19**	**31**	**43**	**55**	**57**	**59**	**61**	**63**	**65**	**67**	**71** [b]
IMMUNOLOGIC STUDIES												
Peripheral Blood Gene Expression Profile – Real Time PCR/Gene Chip	X	X	X	X	X			X				X
Flow Cytometry – Surface Staining	X	X	X	X								
Apoptosis (TUNEL)	X	X	X	X								
Frozen PBMC - ELISPOT	X	X	X	X								
Antigen Specific Antibodies	X	X	X	X	X			X				X
Total and specific IgE	X	X	X	X	X			X				X
Basophil Histamine Release	X	X	X	X								

20 Appendix 5: Nasal Allergen Challenge Methodology

Note: The short ragweed extract provided in this study contains 546 μg/mL *Amb a 1* (during the 2003 treatment and follow up phase) and 368 mcg/ml Amb a1 (during the 2004 extended follow up phase). The pharmacist or designee is required to prepare a diluted solution of the original ragweed extract vial, so that this solution contains approximately 273 μg/mL *Amb a 1* (during the treatment and follow up phase) and 283 μg/mL *Amb a 1* (during the 2004 extended follow up phase) and then follow the procedures below. This diluted solution is considered the "1:10" vial for the intradermal (intracutaneous) skin test.

All participants enrolled in the study will undergo nasal allergen challenge with ragweed. The nasal allergen challenge will comprise four periods: (1) baseline; (2) challenge; (3) immediate phase reaction; and (4) late phase reactions measured 16 to 24 hours after challenge. Any antihistamine, including the rescue medication, is prohibited 72 hours prior to skin test or nasal allergen challenge.

Nasal allergen challenges will be performed before skin testing, since allergy skin testing involves multiple injections of ragweed, which might influence nasal responses. However, the opposite is not true. At baseline, an acoustic rhinogram will be recorded, pulmonary function performed and nasal symptoms assessed. Nasal symptoms include sneezing, itching, and sinus pressure will be assessed on a 0 (none), 1 (mild), 2 (moderate), and 3 (severe) scale. Vehicle control (saline) will be administered to the nose via nasal spray pump. Two sprays will be administered to each nostril (2 sprays = 0.180 mL) for three times. Ten minutes after the third saline administration, acoustic rhinometry and pulmonary function will be performed and nasal symptoms will be assessed. This will be used as baseline. After a third saline control, nasal scrapings will be taken from the inferior and medial turbinates anteriorly using a rhinoprobe. Rhinoprobe samples will be analyzed for cell differentials for inflammatory cells (examining 100 inflammatory cells) and real-time PCR examining for: IL-4, IL-5, IL-13, IL-10, IL-9, TGFβ, IFN-γ, MIP-1α, MIP-1β, and TARC.

Immediately thereafter, the challenge phase will begin which will consist of administering increasing concentrations of ragweed at 1;1,000,000, 1:100,000, 1:10,000, 1:1000, 1:100, and 1:10 (the 1:10 contains approximately 273-283 μg/mL of *Amb a 1*). Ten minutes after each administration of an allergy concentration, an acoustic rhinogram will be recorded, pulmonary function performed and nasal symptoms assessed (sneezing, itching, and sinus pressure). The participant is instructed to blow nose before rhinometry. The challenge will continue until: 1) There is a 30% decrease in nasal volume as determined by acoustic rhinometry, 2) the maximum concentration is reached, or 3) A 20% decrease in FEV_1 is reached. After a 30% decrease in nasal volume is observed, a repeat rhinogram will be performed at 30 minutes after the allergen challenge with recording of nasal volume.

The provocative dose to elicit a 30% decrease in nasal volume (PD_{30}) will be calculated by interpolating the 30% decrease in total nasal volume between ΣA and ΣB, where ΣA=the summation of all doses below the 30% threshold; and where ΣB = ΣA + the dose used to

cross the 30% threshold. "Dose" is calculated by multiplying the *AMB a 1* concentration for each stage by 0.36 (0.36 represents four sprays of 90 microliters to equal 360 microliters of solution administered to the patient at each timepoint.)

The PD30 will be diluted by 4 in order to allow for 4 sprays of 90 microliters to be administered during the challenge.

Once participants have a nasal allergen challenge PD 30 defined, they will receive that dose at every subsequent visit. If no PD 30 is achieved the 1:10 dilution will be administered at subsequent visits .The investigators will assess participants to determine if they have a decrease in nasal allergen challenge-induced nasal volume subsequent to treatment.

Participants will return in 16 to 24 hours for a repeat acoustic rhinometry measure. Scrapings using a rhinoprobe will be taken from the left nares at 16 to 24 hours for a cell differential and RT-PCR as outlined above.

21 Appendix 6: Nasal Nitric Oxide Methodology

Nasal nitric oxide will be performed prior to omalizumab administration and rush immunotherapy administrations using standardized and recommended procedures as published by the American Thoracic Society.[46]

Using a nitric oxide analyzer, air will be entrained through the nasal passage by placing against 1 nares a tube with a silicone seal, through which air will be drawn at rates of 25 mL/second and 50 mL/second. During the nasal air aspiration procedure, the vellum will be closed by closing the lips and maintaining a mouth pressure from the lungs sufficient enough to fully expand the cheeks. A side-stream sample will be taken from the tube and analyzed via chemiluminescence for nitric oxide concentration. A nitric oxide release rate can be calculated and verified with the two rates of collection multiplied by the concentration of nitric oxide observed at each rate. If ambient nitric oxide is greater than 25 ppb, a second tube with a silicone seal will be placed against the opposite nares through which NO-free air will be drawn. Three readings will be measured and the average used.

22 Appendix 7: FcεRI Receptors Measurement and Purification of Enriched Basophils

Human basophils will be enriched from EDTA-anticoagulated venous blood (40 mL) using gradient separation techniques. Viability after purification will be determined by trypan blue dye exclusion. Basophil counts will be performed after staining with alcian blue. In our experience, the purity of basophils ranges from 5% to 18%. If necessary, further purification of basophils will be done by immunomagnetic negative selection with a basophil purification kit (containing a mixture of mAbs recognizing CD3, CD7, CD14, CD15, CD16, CD36, CD45RA, and HLA-DR) and a MidiMACS column (both from Miltenyi Biotec Inc., Auburn, CA).

23 Appendix 8: Sample- Participants Diary Card

PROTOCOL: ITN019AD
SITE: (circle one) 001 002 003
PARTICIPANT ID#:_____

Use the following key to record symptom severity:

0 = NONE Symptoms are absent	**1 = MILD** Symptoms are barely present; not annoying or troublesome	**2 = MODERATE** Symptoms are clearly present and/or bothersome; don't interfere with sleep or daily activities	**3 = SEVERE** Symptoms are hard to tolerate and interfere with daily activities or sleep

DATE: (dd-mmm-yyyy)														
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sneezing														
Rhinorrhea/ runny nose														
Itchy nose, throat, palate														
Itchy, watery red eyes														
Nasal congestion / stuffiness														
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Rescue antihistamine use (# capsules)														
Time spent outdoors (hours)														

Participants will assess symptoms at the same time each morning and each evening (about every 12 hours).

Note: Rescue medication should only be used if significant symptoms are experienced. If you are not sure if your symptoms are significant, contact your study site.

24 Appendix 9: RQLQ Sample

Immune Tolerance Network

Protocol No.	Investigator No.	Participant No.	Participant Initials
ITN019AD	___ ___ ___	___ ___ ___	___ ___ ___

QUALITY OF LIFE QUESTIONNAIRE

Date of Assessment: ____/_____/_____
dd mmm yyyy

Please complete **all** the questions by checking the number that best describes how **troubled** you have been during the **last week as a result of your nose/eye symptoms**.

How **troubled** have you been by each of these activities during the **last week** as a result of your nose/eye symptoms?

ACTIVITIES	Not troubled (0)	Hardly troubled at all (1)	Somewhat troubled (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(1) REGULAR ACTIVITIES AT HOME AND AT WORK (your occupation or tasks that you have to do regularly around your home)	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(2) SOCIAL ACTIVITIES (e.g., activities with your family and friends, playing with children and pets, sex, hobbies)	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(3) OUTDOOR ACTIVITIES (e.g., gardening, mowing the lawn, sitting outdoors, sports, going for a walk)	0☐	1☐	2☐	3☐	4☐	5☐	6☐

How **troubled** have you been by each of these sleep problems during the **last week** as a result of your nose/eye symptoms?

SLEEP	Not troubled (0)	Hardly troubled at all (1)	Somewhat troubled (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(4) Difficulty getting to sleep	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(5) Wake up during the night	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(6) Lack of good night's sleep	0☐	1☐	2☐	3☐	4☐	5☐	6☐

How **troubled** have you been during the **last week** as a result of these symptoms?

NON-NOSE/EYE SYMPTOMS	Not troubled (0)	Hardly troubled at all (1)	Somewhat trouble (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(7) Fatigue	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(8) Thirst	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(9) Reduced productivity	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(10) Tiredness	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(11) Poor concentration	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(12) Headache	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(13) Worn out	0☐	1☐	2☐	3☐	4☐	5☐	6☐

Continued

Final: March 21, 2003 1

Immune Tolerance Network

Protocol No.	Investigator No.	Participant No.	Participant Initials
ITN019AD	___ ___ ___	___ ___ ___	___ ___ ___

QUALITY OF LIFE QUESTIONNAIRE - CONTINUED

How **troubled** have you been by each of these problems during the **last week** as a result of your nose/eye symptoms?

PRACTICAL PROBLEMS	Not troubled (0)	Hardly troubled at all (1)	Somewhat troubled (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(14) Inconvenience of having to carry tissues or handkerchief	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(15) Need to rub nose/eyes	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(16) Need to blow nose repeatedly	0☐	1☐	2☐	3☐	4☐	5☐	6☐

How **troubled** have you been by each of these symptoms during the **last week**?

NASAL SYMPTOMS	Not troubled (0)	Hardly troubled at all (1)	Somewhat troubled (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(17) Stuffy/blocked nose	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(18) Runny nose	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(19) Sneezing	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(20) Post nasal drip	0☐	1☐	2☐	3☐	4☐	5☐	6☐

How **troubled** have you been by each of these symptoms during the **last week**?

EYE SYMPTOMS	Not troubled (0)	Hardly troubled at all (1)	Somewhat troubled (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(21) Itchy eyes	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(22) Watery Eyes	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(23) Sore eyes	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(24) Swollen eyes	0☐	1☐	2☐	3☐	4☐	5☐	6☐

How often during the **last week** have you been troubled by these emotions as a result of your nose/eye symptoms?

EMOTIONAL	Not troubled (0)	Hardly troubled at all (1)	Somewhat troubled (2)	Moderately troubled (3)	Quite a bit troubled (4)	Very troubled (5)	Extremely troubled (6)
(25) Frustrated	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(26) Impatient or restless	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(27) Irritable	0☐	1☐	2☐	3☐	4☐	5☐	6☐
(28) Embarrassed by your symptoms	0☐	1☐	2☐	3☐	4☐	5☐	6☐