

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed–toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006;355:1445-55.

Appendix to appear on the Web

I. Supplemental Table 1:

Study Flow Diagram:

	Study Opened	Enrollment	Received at least one injection	First Post Therapy Visit	End of first period	Enrollment for second period	End of second period
	5/9/2001	5/21/2001-6/19/2001			January 2002	August 2002	October 2002
Placebo Participants		11	11	9	9	9	9
AIC Participants		14	13	10	10	8	6

II. Supplemental Methods Section:

Treatment Assignments:

The treatment assignment list was provided to the designated allergy study technician by the Immune Tolerance Network's statistical and clinical coordinating center. The blinded study coordinator enrolled participants using an internet data entry system, and received a blinded treatment code for each participant upon enrollment. An unblinded technician matched the treatment code to the treatment assignment and prepared study injections before each participant's visit. All injections were administered by blinded study personnel. At all treatment and assessment visits, patients were seen by a blinded physician, the blinded study coordinator, or blinded study personnel.

Guideline of Criteria for Dosage Adjustment:

Adjustment in Dosage – Local Reactions

Dose level adjustments were made based on the evaluation of local reactions following each study injection.

- a) modification for moderate or severe local reactions within 24 hours of injection:

If a dose results in a wheal (swelling) of more than 20mm (mean diameter) within

24 hours of injection, at the next scheduled visit (approximately 7 days later) the dose of AIC was reduced to the last previous dose that did not result in a wheal of more than 20mm (mean diameter).

b) modification for local inflammation or swelling more than 48 hours after the previous injection:

For local inflammation or swelling present more than 48 hours after an injection, the dose was reduced to the previous level.

If local reactions persisted until the next scheduled injection (approximately 7 days later), no further injections were administered until the reactions had resolved. Once the local reactions resolved, the next dose was decreased to the previous level.

Adjustment in Dosage – Systemic Reactions

Examples of injection-related systemic reactions that would require an adjustment in dosage schedule include coughing, wheezing, chest tightness, throat tightness, generalized itching, urticaria, angioedema, flushing, chills, or unusual sweating.

If systemic reactions persisted until the next scheduled injection (approximately seven days later), no further injection was administered until the systemic reactions had resolved. Once the systemic reactions had resolved, the schedule was decreased by two dose levels.

Adjustment in Dosage – Missed Doses

Subjects who missed a single scheduled study injection received the missed dose at the next study visit. If a subject missed two consecutive study injection visits, the dosage was adjusted by repeating the last dose or dose series from the previous study visit. Subjects who missed two or more scheduled study injection visits in a row could be withdrawn from the study at the discretion of the sponsor. The

investigator made all reasonable attempts to follow-up on any patient who had missed 2 or more consecutively scheduled visits.

Reasons for Stopping a Subject From Receiving Additional Injections

- Two respiratory or other clinically significant systemic reactions (e.g. angioedema, generalized urticaria) temporally associated with AIC administration;
- Anaphylaxis (e.g., hypotensive episode requiring medical intervention);
- Severe respiratory reaction or symptoms occurring within two hours of injection;
- Any life-threatening event.

Reasons for Halting the Study

- *Anaphylaxis (e.g., hypotensive episode requiring medication intervention);*
- *Life-threatening event;*
- *Death of a subject*

Sample Size:

The power calculation in the protocol provided justification for the 40 subject sample size. Based on unpublished data on nasal lavage albumin levels (the primary outcome) a sample size of 36 patients was calculated to provide 80% power to detect a difference between the two groups at a single time point with a two-sided t-test under the null hypothesis of equal mean change and an 0.05 significance level. A sample size of 40 provides for 10% dropout in a t-test of those who provide complete data. The intended sample size of 40 participants was not reached because enrollment was terminated in June 2001 when it was believed that the necessary study visits for additional subjects could not be completed before onset of the 2001 ragweed season.

Study Population Inclusion/Exclusion Criteria:

Individuals eligible for enrollment into this study were adult men and women who: were between 18 and 60 years of age; were willing and able to provide written informed consent; had a history of fall, seasonal allergic rhinitis; displayed skin-prick puncture test reactivity to a licensed, standardized

ragweed extract (at least a $\Sigma_E=30\text{mm}$) within 16 weeks prior to study entry; exhibited a positive acute response to nasal challenge with licensed, standardized ragweed extract (defined as ≥ 3 sneezes over the diluent nasal challenge and a 2-fold increase in albumin) within 16 weeks prior to study entry; were in general good health; were available for the duration of the study. Women of childbearing age who were sexually active must have consistently used a highly effective method of birth control (oral contraceptive, IUD, condom plus spermicide, Depo-Provera®) for at least one month prior to study entry and for the duration of the study.

Individuals not eligible to be enrolled into the study were those who: were pregnant or breastfeeding; were asthmatic requiring daily “maintenance” medications or use of “PRN medications” (i.e. albuterol or similar bronchodilators) greater than 2 times per week (1 time use=2 puffs of albuterol) or ever had a hospital admission for asthma; had clinically significant acute or chronic illnesses; had severe symptoms of allergic rhinitis during the spring and summer grass and tree pollen season, or moderate to severe perennial allergies (e.g. cat dander or dust mites); were currently receiving immunotherapy to any allergens or had ever received anti-IgE antibody; had received ragweed immunotherapy within the last 5 years [a limited number of participants (no more than 20% of enrolled participants) with a history of ragweed immunotherapy more than 5 years ago may be enrolled]; had taken systemic corticosteroids or other immunomodulators or immune suppressive medication within month prior to study entry; had taken any antihistamine within 10 days prior to study entry; were not willing or able to comply with all the requirements of the protocol, in the opinion of the investigator; had participated in another investigational trial within 30 days prior to study entry; had a history of anaphylaxis; had a clinically significant abnormality of screening blood chemistry, hematology, or urinalysis; were taking a β -adrenergic blocker (e.g., propranolol) either systemically or topically; had a history of alcohol abuse; had a history of illicit drug use (current or previously); had hypersensitivity to any of the vaccine components other than ragweed; elected to receive non-study vaccine(s) within 30 days of any study injection; were not able to complete the study visits in the period specified before the ragweed

season, or were planning to travel outside of the “ragweed belt” (area of the United States between the Rocky Mountains and the Atlantic Ocean from Connecticut to North Carolina) for more than 14 consecutive days during the ragweed season.

Nasal Challenge Assessment:

Albumin score: For nasal lavage, samples were collected during the nasal challenge procedure at: pre-~~After~~ oxymetazoline, post-diluent, post-ragweed (10 AU, 100 AU, 1000 AU), and post- late phase.

For the primary endpoint, the albumin score was calculated as the ratio of the post-RW challenge (10AU, 100AU, 1000AU, or late-phase) to the post-diluent challenge. Because the albumin results are non-normally distributed, the log (base 10) of each albumin ratio was used.

Clinical Assessments:

During the RW seasons, patients self-assessed their overall hay fever condition using a separate (0-100 mm) Visual Analog Scale (VAS) (16), which was scored on the 7th day of each week from pre-RW to two weeks post-RW season. Patients also kept daily symptom diaries during the RW season, scoring twelve symptoms in three categories: nasal (itchy nose or throat/runny nose/sneezing/nasal congestion or stuffiness); chest (wheezing/chest tightness or constrictive throat sensations/cough/shortness of breath); eye/skin (itching eyes/watering eyes/redness of eyes/itching skin) on a scale of 0 (no symptoms) to 5 (very severe symptoms) (17).

In addition, a standardized rhinoconjunctivitis quality of life questionnaire (RQLQ) (19) was completed before, in the middle and at the end of the RW pollen season. The RQLQ uses 28 questions scored on a 0-6 scale (6 = worse) in 7 mutually exclusive domains (activity/sleep/nasal symptoms/eye symptoms/non-nose and eye symptoms/practical problems/emotional).

Patients were supplied with specific “relief medications” for use only as-needed to relieve moderate-to-severe nasal or eye symptoms that they were unwilling to tolerate [Allegra® (fexofenadine HCL) 60 mg tablet every 12 hours, as needed, for relief of itchy watery eyes, itchy nose, runny nose, sneezing, and postnasal drainage; Sudafed® (pseudoephedrine) 30-60mg every 6 hours, as needed, for relief of nasal congestion or stuffiness]. Participants were instructed not to use other decongestants or antihistamines, intranasal or systemic corticosteroids, or cromolyn. None of the patients in either the AIC or PL groups used an anti-leukotriene agent. Medications were scored both as “weighted” (assigned a point score) and “unweighted” medication use. The “unweighted” score refers to the “raw” compilation of medication use. In calculating the “weighted” use score, each Allegra (60 mg) pill was weighted 2 points, each Sudafed (30 mg) pill was weighted 1 point. A short-acting antihistamine was scored as 1 point/dose, a long-acting antihistamine as 2 points/dose, a short-acting decongestant as 1 point/dose, a combination short-acting antihistamine/ decongestant as 2 points/dose, and a nasal steroid as 1 point/spray.

Laboratory tests monitored for safety included CBC and differential, platelet count, sedimentation rate, serum chemistries, [total serum protein, albumin, globulin, A/G ratio, bilirubin (total, direct, indirect), ALT (SGOT), alkaline phosphatase, GGT, lactic dehydrogenase, urea nitrogen, creatinine, BUN/creatinine ratio, uric acid, calcium, inorganic phosphorus, total cholesterol, triglycerides, glucose, sodium, potassium, chloride, CO₂, magnesium, CPK, C3, C4, ANA, anti-single-stranded and anti-double-stranded DNA antibody titers,] and urinalysis.

Mechanistic Assessments:

Serum anti- Amb a 1- specific IgG and IgE levels were measured via enzyme immunoassays (EIA) (20) at several timepoints, including baseline, post-treatment, pre-season, and both 2 weeks and 2 months following the allergy season (2001) and at the beginning, middle, and end of the second ragweed season (2002). IgE anti-short RW was quantitated in KIu/L using the ImmunoCAP System

(Pharmacia, Kalamazoo, MI). IgG anti-short RW was measured in U/ml using a Solid Phase Radioimmunoassay (1U= 1ng) (20).

The FAP assay is a FACS-based immunoassay which provides a functional readout of antibody response to treatment through measurement of levels of the allergen-specific IgG blocking antibody in serum. The FAP assay detects the ability of IgG in patient serum to inhibit RW allergen binding to low affinity IgE receptors on B cells. It was performed in the laboratory of Stephen S. Durham, M.D. in London UK as described in detail (21).

At various time-points cellular immune responses were measured. PBMCs were isolated from whole blood by Percoll gradient centrifugation ($d = 1.081$ g/ml). Intracellular IL-4 and IFN- γ levels were measured via both 3-color flow cytometry and ELISA in Amb a1 activated CD4⁺ CD69⁺ T cells (as well as Tetanus toxoid activated and controls) using previously described methods (21). Basophil IL-4 measurements were assessed by 2-color flow cytometry. In brief, PBMCs were stimulated with Amb a1 (50 ng/ml) or controls in the presence of brefeldin A (1 μ m). After 16 hours, cells were harvested, and fixed as described (22).

Peripheral blood was collected into Paxgene (Becton-Dickinson) vacutainers for later purification of RNA. RNA was subsequently purified by Qiagen RNA-easy kit and analyzed for concentration and purity using an Agilen Bioanalyzer. Total RNA was shipped to Applied Biosystems for assessment of gene expression profiles of 184 cytokine, chemokine and other immune response genes, using quantitative real-time PCR. An additional pre-amplification step was performed, whereby, total RNA was incubated with all 184 primer pairs and amplified for 10 rounds of PCR prior to individual real time PCR reactions using the ABI standard methodology.

Statistical Analysis:

The primary and secondary clinical analyses were completed based on an analysis plan that was pre-defined. Modifications to the analysis plan for clarity or to add additional secondary analyses were documented in memoranda. In the complete study report statistical analysis plan, three types of data sets were included in the analyses: 1) all randomized participants (n=25); 2) completed cases; 3) efficacy subset of cases who reached target dose (or were controls). Only the analysis of the albumin endpoint used the first type of dataset, all randomized participants with imputation for those who did not contribute to this endpoint. Most analyses used complete cases, and some analyses used the efficacy subset containing the target cases. The number of participants in the completed cases or efficacy subset depends on the outcome under analysis.

Descriptive statistics were provided for most outcomes. Treatment group differences in albumin, the primary outcome, were tested using regression modeling. For other endpoints including serum immune response, cellular immune response, skin test outcomes, diary card symptom scores, medication usage, and nasal challenge symptom score, treatment group differences in raw scores and difference from baseline scores were tested using the Wilcoxon rank sum statistic. Treatment group differences in percent change from baseline scores for serum immune response were also tested using the Wilcoxon rank sum statistic. Within group changes for serum immune data were tested using the Wilcoxon signed rank statistic. Post-baseline RQLQ scores were tested using ANCOVA modeling. Repeated measures models were also used for many of these outcomes. Repeated measures models included main effects of treatment, time, and treatment-by time interactions (as applicable); if a pre-treatment value was available it was included as a baseline covariate.

The protocol specified 0.05 for a significance level for the primary outcome and 0.01 for a significance level for all secondary outcomes. This was an exploratory study, and a large number of analyses were completed; nominal p-values are reported, unadjusted for the number of tests completed. Additionally,

analyses included overlapping data (raw scores and difference-from-baseline scores, weighted and un-weighted scores, full season and peak season scores, non-repeated measures and repeated measures analyses). All p values are two sided.

The serum immune response data were updated after preliminary analyses were completed, as part of quality assurance methods; however, the lab conducting these updates remained masked to the preliminary results. Study results from the 2001 season were provided before the end of the study; however, the study coordinator did not review the results and remained blinded to participant treatment status. Several of the endpoints were evaluated and reported before the study completion; the study coordinator remained blinded.

There were no interim analyses for the purpose of stopping the study. Pre-defined study stopping rules based on adverse events included anaphylaxis, life-threatening events, or death of a study participant.

III. Supplemental Results Section:

Supplemental Table 2: Patient Demographics for Patients Enrolled in Second Year:

	For all enrolled		For those enrolled in 2 nd season	
	AIC	PLACEBO	AIC	PLACEBO
# of Patients	14	11	8	9
Male	6 (43%)	4 (36%)	4 (50%)	3 (33%)
Female	8 (57%)	7 (64%)	4 (50%)	6 (67%)
Caucasian	9 (64%)	7 (64%)	6 (75%)	6 (67%)
African American	4 (29%)	4 (36%)	2 (25%)	3 (33%)
Asian	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mixed	1 (7%)	0 (0%)	0 (0%)	0 (0%)
Age [mean] (sem)	37.6 (2.7)	41.7 (2.6)	42.0 (3.8)	42.1 (3.1)
[median] (range)	38 (23-58)	39 (33-60)	42 (25-58)	39 (33-60)
Seasonal Rhinitis (Years) [mean] (sem)	26 (4)	33 (3)	31 (6)	34 (3)
IgE [RW] (mean) (sem)	12.0 (2.8)	9.09.8 (3.5)	14.1 (4.4)	10.8 (4.1)
(median)	9.0	7.0	12.8	7.0

Puncture Skin Test Sensitivity (mm) [median] (range)	72 (47-98)	72 (50-86)	74 (52-93)	72 (59-86)
Other Sensitivity (#)				
Grass	11 (79%)	11 (100%)	7 (88%)	9 (100%)
Trees	11(79%)	11(100%)	7 (88%)	9 (100%)
Dust Mites	10 (71%)	6 (55%)	7 (88%)	5 (56%)
Mold	10 (71%)	6 (55%)	6 (75%)	4 (44%)
Cat	9 (64%)	7 (64%)	5 (63%)	6 (67%)
Dog	6 (43%)	4 (36%)	3 (38%)	3 (33%)
Cockroach	1 (7%)	0 (0%)	0 (0.0%)	0 (0.0%)
Co-morbid Conditions (#)				
Asthma	4 (29%)	2 (18%)	2 (25%)	1 (11%)
Conjunctivitis	13 (93%)	11 (100%)	7 (88%)	9 (100%)
Sinusitis	2 (14%)	4 (36%)	1 (13%)	3 (33%)
Eczema	3 (21%)	1 (9%)	2 (25%)	1 (11%)

There were no statistically significant differences between AIC and placebo groups in these characteristics for those who participated in the second season.

Antibody Results:

AIC induced an increase in Amb a 1-specific IgG in 10/11 patients, with mean titers increasing from 190.2 +/- 40.6 (SE) U/mL at baseline (BL) to 491.6 +/- 180.1 U/mL post-treatment [median 140.7 to 214.8; with a median difference score of 98.8 (p= 0.002)]; however, this response was transient [mean: 356.5 +/- 100.4 U/ml at 2 months post 2001 RW season] and returned to BL levels in the end of the 2002 RW season [mean:168.2 +/- 22.2 U/ml]. Similarly, AIC induced a 2.4-fold increase in RW-specific IgG titer [pre tx (mean): 270.2 +/- 97.0 U/ml; post tx: 642.3 +/- 208.8 U/ml, with a median difference score of 69.3 (p= 0.003)]. No significant post-treatment rise in Amb a 1- or RW-specific IgG titers was observed in the PL-injected group.

Skin Test Sensitivity Results:

Puncture skin test erythema in the AIC group was reduced by 22% [median AIC baseline:72mm, post-treatment:56mm; PL baseline:72mm, post-treatment:76mm] but the change was not statistically different by treatment group (median of change score: AIC:-19; PL:1.0; p_{dfb}=0.12) [NOTE: This p-value is the difference between AIC and placebo, not the test for whether there was a change from

baseline within the AIC group.] In AIC-treated patients, the late-phase intradermal skin test, read at 24 hours, had a greater decrease in erythema size from baseline than in PL patients [median of change score: AIC: -19.5mm, PL: -6.5mm; $p_{dfb}=0.50$].

Functional Antibody Presentation (FAP Assay):

The plots for the FAP assay for all patients studied over the time intervals specified in the Protocol are provided in Supplemental Figure 1A/B.

PBMC IL-10 Data:

The data are plotted in Supplemental Figure 2.

Basophil IL-4 Expression:

The data are plotted in Supplemental Figure 3.

Gene Chip Studies for Novel Genes Modulated by AIC:

Genes were analyzed for differential expression between baseline and subsequent time-points of blood collection. In addition, comparisons were made directly between pre-RW season versus post-RW season for both treated and control groups.

- Peripheral blood samples from 9 subjects (5 in treatment cohort and 4 in control cohort) were collected at 7 time points over two years; RNA was isolated and analyzed by RT-PCR.
- Based on available data, the outcomes of interest were:
 - Change in expression from pre-ragweed to post-ragweed season in both years of the trial;
 - Change in expression from pre-ragweed to mid-ragweed season in Year 2

of the trial.

- Genes for which this change was different across treatment groups, as defined by any p-value < 0.10, have been tabulated.

While several interesting genes emerged as potentially differentially expressed, the magnitude of the change, combined with the small number of patients studied in this assay, do not allow us to make any definitive statements. However, the data set is provided for review:

Supplemental Table 3: Gene Chip Studies:

Year 1: change from pre-ragweed to post-ragweed season

Gene	Avg Ct, Tx	Av Ct, Ctrl	Diff	p-value
INFGR1	0.82	0.40	0.41	0.097

Year 2: change from pre-ragweed to mid-ragweed season

Gene	Avg Ct, Tx	Av Ct, Ctrl	Diff	p-value
IL1B	0.56	-0.27	0.83	0.069
CSF2RB	0.50	-0.19	0.69	0.070
G1P3	0.02	-1.08	1.10	0.076
IRF7	-0.06	-0.95	0.88	0.090
IL4R	0.37	-0.31	0.68	0.090
IL8RB	0.56	-0.12	0.68	0.094
IL18RAP	0.08	-0.52	0.59	0.095

Year 2: change from pre-ragweed to post-ragweed season

Gene	Avg Ct, Tx	Av Ct, Ctrl	Diff	p-value
GPR44	0.37	-0.47	0.84	0.013
IL2RA	0.40	-0.26	0.66	0.014
CXCR6	0.58	-0.05	0.53	0.082
CCR3	0.29	-0.44	0.73	0.086
IL16	0.23	-0.32	0.55	0.092

Supplemental Table 4: Additional Web Table that Highlights the Statistical Findings:

Selected Non Repeated Measures Analyses

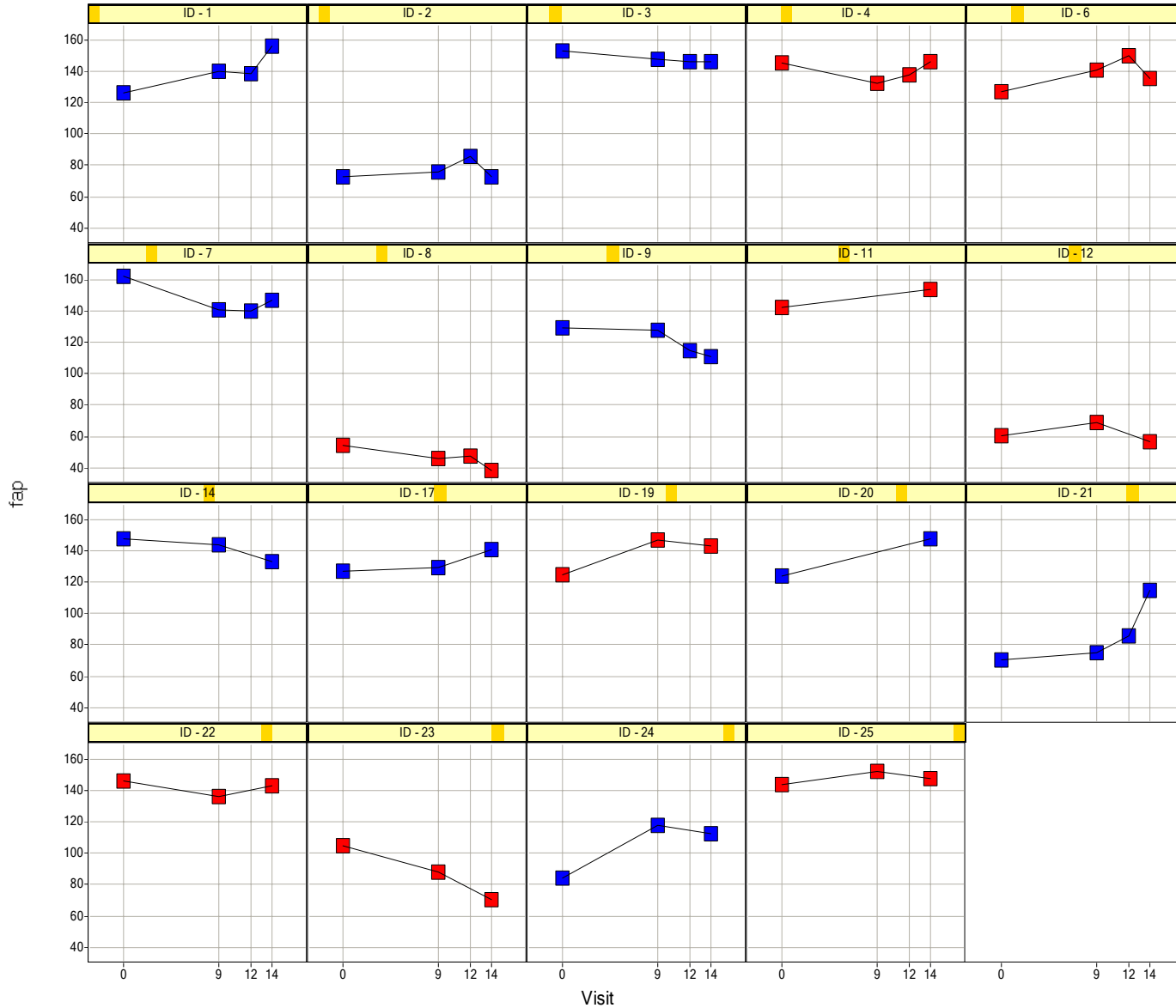
Outcome	PLACEBO						AIC						p-value ¹
	N	med	min	max	mean	Std Err	N	Med	Min	Max	Mean	Std Err	
2001													
RWNasal Challenge													
maximum change in albumin, post-therapy ²	11	2.0	0.1	4.0	2.2	0.3	14	1.7	-1.1	4.9	1.7	0.3	0.47 ²
total challenge score post therapy	9	70.0	22.0	104.5	70.5	9.3	10	37.1	22.8	77.0	41.6	5.6	0.03
total nasal challenge score, post therapy	9	60.0	6.0	89.3	57.8	9.1	10	32.9	14.8	55.0	32.7	4.3	0.02
post nasal drip, post therapy	9	57.0	10.0	87.0	58.2	8.2	10	24.0	0.0	57.0	25.6	6.5	0.007
nasal stuffiness, post therapy	9	69.0	1.0	92.0	57.6	10.8	10	27.5	2.0	67.0	32.7	6.4	0.06
nasal itch, post therapy	9	59.0	3.0	90.0	55.8	11.0	10	27.5	5.0	58.0	31.4	6.2	0.09
sneeze count, post therapy dfb	9	3.0	0.0	7.0	3.3	0.9	10	-1.5	-17.0	7.0	-3.0	2.4	0.03
histamine, post therapy	9	2.2	0.5	15.8	5.3	2.0	10	1.5	0.6	27.0	4.2	2.6	0.40
Symptom Scores													
overall RQLQ, midseason	9	1.6	0.0	3.4	1.6	0.4	9	0.4	0.0	1.4	0.5	0.2	0.05 ³
eye domain, RQLQ midseason	9	1.8	0.0	4.3	1.8	0.5	9	0.3	0.0	2.0	0.5	0.2	0.03 ³
sleep domain, RQLQ midseason	9	3.0	0.0	5.0	2.1	0.6	9	0.0	0.0	1.0	0.3	0.1	0.04 ³
nasal domain, RQLQ midseason	9	2.5	0.0	4.8	2.2	0.5	9	1.0	0.0	2.0	0.9	0.3	0.06 ³
activity domain, RQLQ midseason	9	1.3	0.0	3.7	1.4	0.4	9	0.0	0.0	1.7	0.4	0.2	0.07 ³
RW Skin Test													
Puncture skin test, erythema, post therapy	10	76.0	35.0	111.0	72.3	7.2	10	55.5	38.0	73.0	56.3	3.5	0.07
Puncture skin test, wheal, post therapy	10	15.0	10.0	21.0	15.4	1.0	10	13.0	8.0	18.0	12.8	1.1	0.08
2002													
Symptom Score													
mean full season hayfever VAS score	9	51.3	5.8	79.4	43.7	8.5	7	18.0	1.1	40.4	17.2	5.0	0.05
mean peak season hayfever VAS scale	9	61.2	2.0	89.2	47.2	9.9	7	17.6	1.8	36.8	17.9	4.8	0.05

¹Tested by Wilcoxon Rank Sum, unless otherwise indicated

²imputation used, descriptive statistics are from all imputations, SE is SE of mean; tested with regression model

³ANCOVA model included pre-season score and treatment group as covariates

FAP: All Patients Over Time

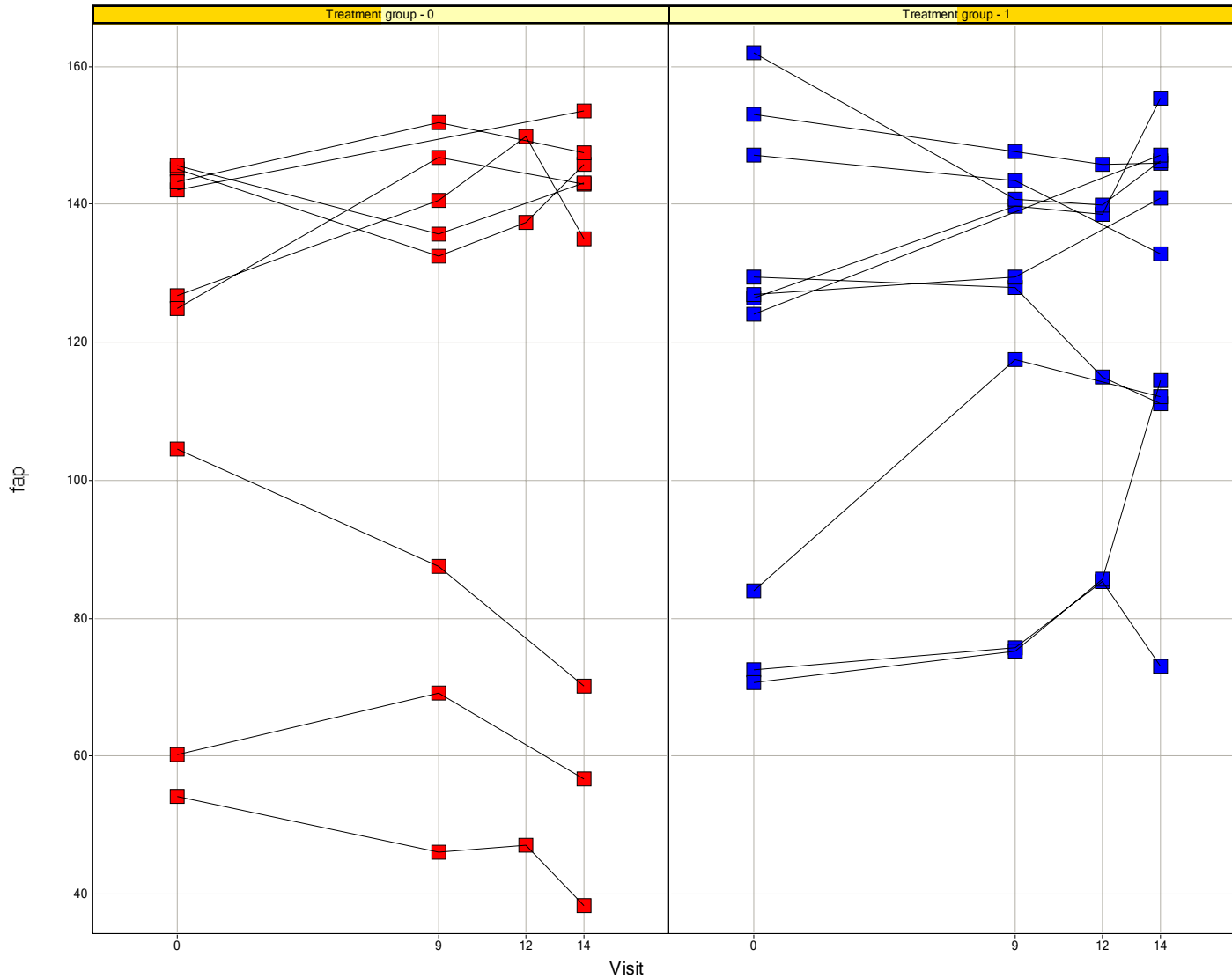


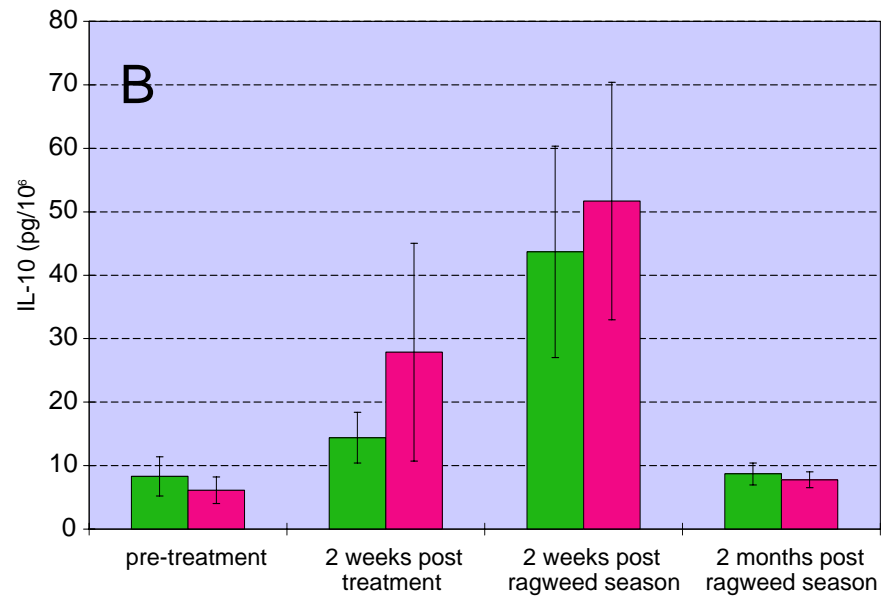
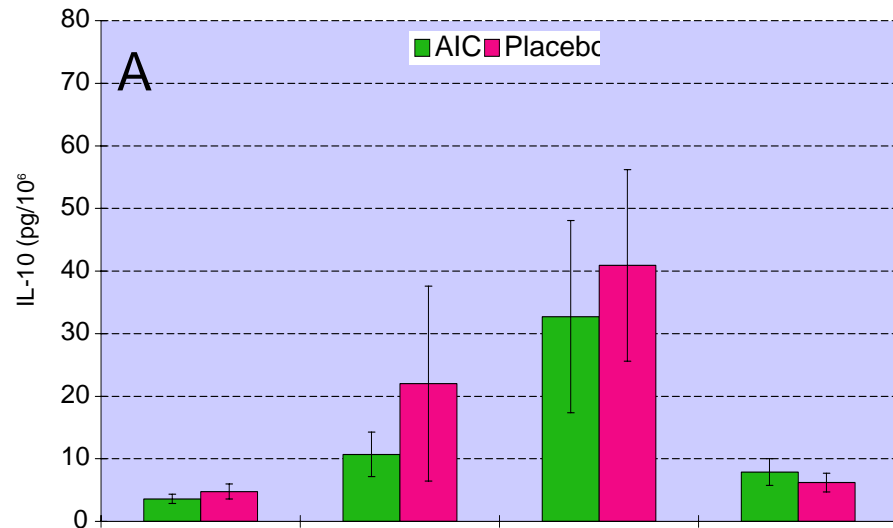
Red = placebo
Blue = AIC

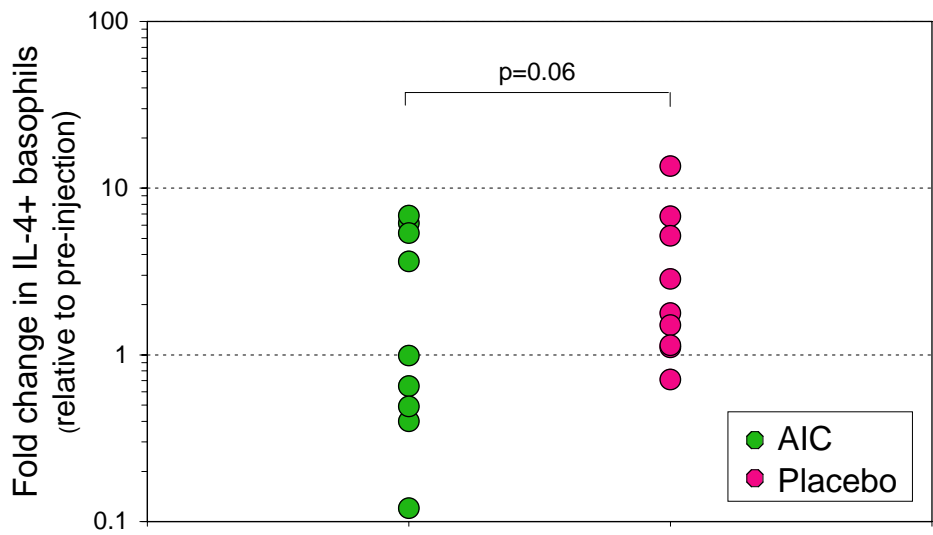
FAP: By Treatment Group

Placebo

Treated







IV. Supplemental Background Studies:

Numerous chemical modifications and physical conjugates of allergens have been devised and tested, but clinical studies of these compounds have failed to provide evidence for an allergen construct that possesses an improved risk:benefit ratio. Listed are citations for a number of the investigational compounds that we, and others, have studied:

1. Lichtenstein LM, Norman PS. and Winkenwerder W.L. Antibody response following immunotherapy in ragweed hay fever: allpyral vs. whole ragweed extract. *J. Allergy*. 1968; 41:49-57.
2. Norman PS, Winkenwerder WL, Lichtenstein LM. Trials of alum-precipitated pollen extracts in the treatment of hay fever. *J. Allergy Clin. Immunol.* 1972;50:31-44.
3. King TP, Norman PS, Tao N. Chemical modifications of the major allergen of ragweed pollen, antigen E. *Immunochemistry*. 1974;11:83-92.
4. King TP, Kochoumian L, Ishizaka K, Lichtenstein LM, Norman PS. Immunochemical studies of dextran coupled ragweed pollen allergen, Antigen E. *Arch. Biochem. Biophys.* 1975;169: 464-473.
5. Norman PS, Lichtenstein LM. Comparisons of alum-precipitated and unprecipitated aqueous ragweed pollen extracts in the treatment of hay fever. *J. Allergy Clin. Immunol.* 1978;61:384-389.
6. Norman PS, Ishizaka K, Lichtenstein LM, Adkinson NF, Jr. Treatment of ragweed hay fever with urea-denatured antigen E. *J. Allergy Clin. Immunol.* 1980;66:336-341.

7. Norman PS, Lichtenstein LM, Marsh DG. Studies on allergoids from naturally-occurring allergens. IV. Efficacy and safety of long-term allergoid treatment of ragweed hayfever. *J. Allergy Clin. Immunol.* 1981;68:460-470.
8. Norman, PS, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J. Allergy Clin. Immunol.* 1982;70:248-260.
9. Norman, PS, King TP, Alexander JF, Jr., Kagey-Sobotka A, Lichtenstein LM. Immunologic responses to conjugates of Antigen E in patients with ragweed hay fever. *J. Allergy Clin. Immunol.* 1984;73:782-789.
10. Bousquet J, Hejjaou A, Skassa-Brociek W, et al. Double-blind placebo controlled immunotherapy with mixed grass-pollen allergoids. 1. Rush immunotherapy with allergoids and standardized orchard-grass pollen. *J. Allergy Clin Immunol.* 1987;80:591-598.
11. Grammer LC, Shaughnessy MA, Finkle AM, Shaughnessy JJ, Patterson R. A double-blind, placebo controlled trial of polymerized whole grass administered in an accelerated dosage schedule for immunotherapy of grass Pollinosis. *J. Allergy Clin Immunol.* 1986;78:1180-84.
12. Norman PS, Ohman JL Jr, Long AA, et al. Treatment of cat allergy with T cell reactive peptides. *Am J. Respir Crit Care Med.* 1996;154 (Pt 1):1623-8.