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Original article

SQ-standardized sublingual grass immunotherapy: Confirmation of disease-modification 2 years after 3 years of treatment in randomized trial

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Abstract

Background: The main aim of specific immunotherapy is sustained effect due to changes in the immune system that can only be demonstrated in long-term trials.

Objective: To investigate sustained efficacy and disease-modification in a 5 year double-blind, placebo-controlled trial, including 2 years of blinded follow-up after completion of a 3-year period of treatment, with the SQ-standardized grass allergy immunotherapy tablet (AIT), Grazax® (*Phleum pratense* 75,000 SQ-T/2,800 BAU[1], ALK, Denmark) or placebo.

Methods: A randomized, double-blind, placebo-controlled, multinational, phase III trial including adults with a history of moderate-severe grass pollen induced allergic rhinoconjunctivitis, with or without asthma, inadequately controlled by symptomatic medications. 238 participants completed the trial. Endpoints included rhinoconjunctivitis symptom and medication scores, combined scores, asthma symptom and medication scores, quality of life, days with severe symptoms, immunological endpoints, and safety parameters.

Results: The mean rhinoconjunctivitis daily symptom score was reduced by 25-36% ($p \leq 0.004$) in the grass AIT group compared to the placebo group over the 5 grass pollen seasons covered by the trial. The rhinoconjunctivitis daily medication score was reduced by 20-45% ($p \leq 0.022$ for seasons 1-4; $p=0.114$ for season 5) and the weighted rhinoconjunctivitis combined score were reduced by 27-41% ($p \leq 0.003$) in favor of active treatment. The percentage of days with severe symptoms during the peak grass pollen exposure was in all seasons lower in the active group than in the placebo group, with relative differences of 49-63% ($p \leq 0.0001$). Efficacy was supported by long-lasting significant effects on the allergen-specific antibody response. No safety issues were identified.

[1]SQ-T (standardized quality tablet units) and BAU (biological activity units) are quantitative measures of biological activity; i.e. the potency of allergen extracts/vaccines. One grass AIT contains 75,000 SQ-T of Timothy (*Phleum pratense*) grass pollen extract (measure of total biological potency using ALK in-house reference), equivalent to 2,800 BAU (measure of total biological potency, defined by the FDA).

Conclusion: The results confirm disease-modification by SQ-standardized grass AIT in addition to effective symptomatic treatment of allergic rhinoconjunctivitis.

Clinical implications

Grass allergy immunotherapy tablet treatment has sustained, disease-modifying efficacy 2 years after completion of treatment and represents an important treatment option in grass allergy inadequately controlled by symptomatic medications.

Capsule summary

The first report of a full 5-year randomized, placebo-controlled trial of grass allergy immunotherapy tablet for seasonal allergic rhinitis, confirming effective symptom control and disease-modification 2 years after completion of 3 year's treatment.

Keywords

Allergy immunotherapy tablet, disease-modification, grass pollen, immunotherapy, sublingual, sustained efficacy, placebo-controlled, *Phleum pratense*, rhinoconjunctivitis, rhinoconjunctivitis quality of life

Abbreviations

AIT: allergy immunotherapy tablet, SQ: standardized quality, DSS: daily symptom score, DMS: daily medication score, RCS: rhinoconjunctivitis combined score

Introduction

The increasing prevalence of atopic diseases such as allergic rhinitis/rhinoconjunctivitis, allergic asthma, and food allergy is a major health issue worldwide. In Western Europe and the US up to 20% of the adult population suffers from allergic rhinoconjunctivitis[1-3]. Indirect costs such as absenteeism from work and decreased productivity are substantial; estimates suggest 3.5 million lost workdays per year in the US alone[4].

The main aim of specific immunotherapy is a sustained significant and clinically relevant disease-modifying effect in post treatment years[5]. Changes in T-cell reactivity and induction of non-IgE antibodies with blocking capacity are regarded as immunological markers of the immunomodulation that leads to clinical tolerance[6;7].

It is generally accepted that the appropriate primary endpoints for assessing the response of allergic rhinoconjunctivitis to specific immunotherapy are the rhinoconjunctivitis symptom and medication scores, which may be reported separately or in a combined score. Because both symptom and medication scores are reduced by effective treatment of allergic rhinoconjunctivitis, it is now considered advantageous by global regulatory bodies to report the 2 responses in a single combined score[5;8;9].

The SQ-standardized grass allergy immunotherapy tablet (AIT) contains an extract of *Phleum pratense* (Timothy grass) pollen. The grass AIT is indicated and approved in most of Europe for disease-modifying treatment of grass pollen induced rhinitis and conjunctivitis in adults and children. The tolerability and efficacy of the tablet has been demonstrated in several randomized, placebo-controlled trials in Europe and North America[10-16].

115

116 This is the first full 5-year double-blind, placebo-controlled trial demonstrating efficacy of
117 sublingual tablet immunotherapy with 3 years of treatment and 2 years of immunotherapy-free
118 follow-up after completion of treatment. Symptomatic medications were provided to all participants
119 as needed throughout the trial.

Methods

Clinical trial design

Details of the randomized, double-blind, placebo-controlled trial, conducted according to the Declaration of Helsinki[17], have been published previously[11;12;14] (ClinicalTrials.gov number: NCT00227279). From original 1 year of treatment, the trial was extended to cover in total 3 years of active treatment and 2 years of follow-up to investigate long-term and sustained efficacy of grass AIT (extension implemented in April 2005). The ethics committees in each country approved the trial as well as the extension, and participants gave written informed consent and re-consented to the extension prior to its inception. Enrollment of participants commenced in September 2004. A total of 51 sites in 8 European countries participated in the trial. Data collection, management, statistics and results reporting upon trial completion were performed by the sponsor.

Trial population

The main inclusion criteria were: males or females; aged 18-65 years; a clinical history of grass pollen induced allergic rhinoconjunctivitis of 2 years or more requiring treatment during the grass pollen season, with rhinoconjunctivitis symptoms interfering with usual daily activities or sleep and remaining troublesome despite treatment with symptomatic medications; and positive skin prick test (wheal diameter ≥3 mm) and serum specific IgE (IgE CAP class ≥2) to *Phleum pratense*. The main exclusion criteria were: FEV_1<70% of predicted value; a clinical history of symptomatic seasonal allergic rhinitis/asthma due to tree or weed pollen potentially overlapping the grass pollen season; a clinical history of significant active perennial allergic rhinitis/asthma caused by an allergen to which the participant was regularly exposed; previous immunotherapy within the last 5 years; and a history of anaphylaxis or angioedema.

144 **Assignment and treatment**

145 Block randomization, randomly assigned participants to daily treatment with grass AIT (Grazax®,
146 *Phleum pratense* 75,000 SQ-T/2,800 BAU, ALK, Denmark) or placebo (1:1). Randomization was
147 performed by ALK (by a statistician not otherwise involved in the trial) using the SAS® system for
148 Windows (version 8e).
149
150 The tablets were supplied as fast-dissolving, neutral-tasting oral lyophilisates for sublingual
151 application. Excipients included gelatin, mannitol and sodium hydroxide. Placebo was
152 indistinguishable from the active tablet in appearance but contained no grass pollen extract.
153 Investigational treatment was initiated 4-8 months prior to the anticipated start of the grass pollen
154 season 2005 and per the extension continued in a double-blinded manner until the end of the season
155 2007. The additional 2 years of follow-up without investigational treatment was continuously
156 doubled-blinded. During each grass pollen season, all participants had free access to open-labeled
157 symptomatic medications in case of rhinoconjunctivitis or asthma symptoms. The participants
158 attended the clinics at least twice a year; 2 weeks before the anticipated start and 1 week after the
159 grass pollen season.
160
161 **Grass pollen season**
162 Grass pollen counts were obtained from regional pollen stations in each country. The season was
163 defined with start as the first day of 3 consecutive days with grass pollen count ≥10 grains/m^3, and
164 stop as the last day in the last occurrence of 3 consecutive days with pollen count >10 grains/m^3.
165 The peak pollen season was defined as the 15 days period with the highest average pollen count.
166 Cumulated pollen loads were calculated after 3 and 10 weeks of each season.
167

Outcomes

The main objective was to evaluate sustained efficacy 2 years after completion of a 3-year period with active treatment compared to placebo. The ranked co-primary endpoints each year were average rhinoconjunctivitis daily symptom score (DSS) and rhinoconjunctivitis daily medication scores (DMS) within the grass pollen seasons. The scores were registered daily from the pre-seasonal visit and until the post-seasonal visit in an electronic diary (LogPad, PHT Corporation, Charlestown, US). A weighted rhinoconjunctivitis combined score (RCS) was calculated based on the primary endpoints (please refer to the Online Repository at www.JACIonline.org for details).

Further secondary endpoints included rhinoconjunctivitis quality of life[18] during the peak grass pollen seasons, percentages days with severe symptoms (defined as a symptom score of 3 in any of the 6 rhinoconjunctivitis symptoms), change from baseline in specific IgG_4 and IgE-blocking factor (i.e. the presence of components blocking IgE-allergen binding); change from baseline in facilitated allergen presentation (FAP) inhibition (for details on immunological methods: see Online Repository at www.JACIonline.org), and safety and tolerability (adverse events, serum and urine safety parameters).

Asthma DSS and asthma DMS were analyzed in the subgroup of participants having asthma at randomization (see Online Repository at www.JACIonline.org for details). Post hoc the asthma combined score was calculated based on the same principle as the RCS.

Statistics

The sample size calculation applied to the first year analysis[11] and no formal power calculation was performed for the extension. Pre-specified data analysis was carried out based on the full

analysis set according to the statistical analysis plan (prepared before unblinding). No imputation of data was done in case of missing data and all available data was used to its full extent. Due to a prospective hierarchical ranking of primary and key secondary endpoints, no adjustments for multiplicity were applied. All endpoints were tested on a 5% significance level and all tests and confidence intervals were 2-sided. The null hypothesis was no difference between the 2 treatment groups.

For the co-primary endpoints (1: rhinoconjunctivitis DSS and 2: rhinoconjunctivitis DMS), the comparison of the 2 treatment groups was done via a generalized linear mixed model. The response variable per participant was the average rhinoconjunctivitis DSS or DMS for the entire grass pollen season. Treatment group was included as a fixed effect. Centre and pollen region were aggregated to country and included as random effect to adjust for variation due to differences in exposure and possible centre effects. Furthermore, estimates were adjusted for different error variation for each treatment group. The residuals were assumed to be normally distributed.

The RCS for the entire season for all 5 years was analyzed using a repeated measurement model with treatment group and a treatment:year interaction as fixed effects. Participant was included as random effect. Confidence intervals for the percentage difference relative to placebo for the adjusted means and medians were found by bootstrapping ($N_{resamples}$=10,000).

For details on the methodology for the additional analyses, please refer to the Online Repository at www.JACI.org.

215 Calculations were performed with the use of SAS statistical software, version 9.2 (SAS Institute,
216 North Carolina, US) and TIBCO Spotfire S+® 8.1 for Windows (TIBCO Software Inc., Palo Alto,
217 US).

Results

Efficacy

At randomization 634 participants were included in the trial (316 active, 318 placebo). 546 participants completed the first season of the trial. When the trial was extended with another 4 years, 195 participants chose not to enroll or were not offered enrolment due to closure of sites. Thus, 351 participants continued in the extension (189 active, 162 placebo). These participants were a representative subset of the population originally included in the trial (see Dahl et al.[12]). 238 participants (135 active, 103 placebo) completed the total 5 years of the trial. A complete participant disposition during the 5 years of the trial is shown in Figure E1 in the Online Repository (www.jacionline.org).

Overall demographics were similar between the 2 treatment groups (please refer to the Online Repository for more details).

The mean rhinoconjunctivitis DSS in each season was reduced by 25-36% in the grass AIT group relative to the placebo group over the 5 grass pollen seasons covered by the trial (Table 1). The rhinoconjunctivitis DMS in each season was reduced by 20-45% (Table 1).

The RCS was reduced by 27-41% relative to placebo in the 5 grass pollen seasons (see Figure 1). The treatment effect was significant for all 5 years of the trial ($p<0.01$ for all years), with a statistically insignificant interaction between treatment and year ($p=0.60$), meaning that the efficacy in terms of the RCS was similar during the 3 treatment seasons and 2 follow-up seasons. The average treatment effect over the 5 seasons was 33% ($CI_{95\%}$ [30%; 38%]) (see Figure 1). The corresponding median value for the average treatment effect was 37% ($CI_{95\%}$ [30%; 42%]).

242 Different approaches to combine the rhinoconjunctivitis DSS and DMS all resulted in statistically
243 significant reductions in the 2nd follow-up year in favor of grass AIT treatment (data not shown).
244
245 The RCS was dependent on the exposure to grass pollen, and with a steeper increase for the placebo
246 group than the active group (see Figure 2). The difference between the grass AIT and the placebo
247 group increased with increasing grass pollen counts.
248
249 The grass pollen seasons varied considerably over the 5 years of the trial, with a significantly lower
250 grass pollen exposure during the 2nd follow-up season (see Figure 3A). The median grass pollen
251 exposure in the 2nd follow-up season was 38% lower than in the 1st treatment season and 30% lower
252 than in the 1st follow-up season and the cumulative exposure during the first 3 weeks of the season
253 30% and 39% lower, respectively. Interestingly, the relative treatment effect on the RCS during the
254 5 seasons covered by the trial was highly correlated to the cumulative pollen exposure in the
255 beginning of the season (see Figure 3B).
256
257 The percentage of days with severe symptoms during the peak grass pollen season was, in all
258 seasons covered by the trial, lower in the active group than in the placebo group with relative
259 differences of 49-63% (Table 2). The risk of having days with severe symptoms during the peak
260 season was 2-3 times lower in the grass AIT group than in the placebo group (odds ratios of 0.47-
261 0.34; Table 2). The participants quality of life, investigated by means of the RQLQ[18], was
262 significantly improved by active treatment (relative differences of 25-32%; Table 2).
263
264 The relation between numbers of days with severe symptoms per week and the reported weekly
265 RQLQ was investigated. For all participants and all seasons there was a good agreement, i.e.

whether in the active group or in the placebo group, the more days with severe symptoms during a week were reflected in a worse quality of life score during that week (see Figure 4, -Δ-). The risk (odds ratio) of having one or more days with severe symptoms in the active group was significantly lower than in the placebo group (see Figure 4, -●-).

The weighted asthma combined score for participants with grass pollen induced asthma at inclusion (N_{active}=79; $N_{placebo}$=72) was reduced by 39% in the active group relative to placebo over the entire grass pollen seasons (p=0.049) and by 44% over the peak seasons (p=0.030), when combining all 5 years.

Immunological changes were assessed by means of difference between active and placebo in change from baseline of specific IgG4, specific IgE-blocking factor and FAP inhibition. The differences between grass AIT and placebo were significant at all assessments, i.e. $p<0.05$ (see Figure 5) and with sustained significant treatment effects at both 1 and 2 years after completion of the 3 years of treatment.

Safety and tolerability

No safety issues in relation to the trial treatment were reported. However, the treatment did cause local application site related adverse events. The 4 most common adverse events were oral pruritus (reported by 44% from the grass AIT group, 4% from the placebo group), mouth edema (19% grass AIT, 1% placebo), throat irritation (13% grass AIT, 2% placebo), and ear pruritus (12% grass AIT, 1% placebo). The majority of these events occurred in the active group, was primarily mild in severity, and had causality most often assessed as related to treatment. Remarkably, the local events occurring in the placebo group were also commonly assessed as related to treatment. The onset of

the common local adverse events in the active group was largely on the first or second day of treatment (see Figure 6: “1 day” and “2 days”), and duration was short (typically for 5-10 min after tablet intake for 2-8 weeks).

No treatment-related serious adverse events or events of severe systemic allergic reactions were reported during the 5 years of the trial. A total of 41 adverse events led to trial discontinuation for 29 participants (18 active, 11 placebo including 1 death). In the grass AIT group 93% of the events leading to discontinuation were assessed as treatment-related, whereas in the placebo group 35% were assessed as treatment-related (for more details see Table E2 in the Online Repository).

No safety issues were detected in lung function assessments, physical examinations, vital signs or safety laboratory analyses.

Discussion

This is the first full 5-year, double-blind, multinational, placebo-controlled trial showing sustained clinical efficacy and disease-modification 2 years after completion of 3 years of treatment with grass AIT. The 2nd follow-up and final year of this trial showed a statistically significant and clinically relevant sustained efficacy on the rhinoconjunctivitis symptom score of 25% (p=0.004) in the active group relative to placebo. This sustained efficacy was observed in the face of the lowest annual cumulative pollen counts observed during the 5 years of the trial, a circumstance known to compromise the ability to detect a significant seasonal treatment effect. The significant reduction in symptoms was accompanied by less use of symptomatic medications. The lack of a statistically significant difference for the medication score during the 2nd follow-up season was probably a consequence of both the low pollen exposure and the reduced sample size. A significant reduction in RCS was obtained during the 5th season, with an average reduction in RCS of 33%, $CI_{95\%}$ [30%; 38%] over the 5 years of the trial. The clinical efficacy of the grass AIT has recently been confirmed in North American populations[15;16].

The levels of rhinoconjunctivitis symptoms and the use of symptomatic medications rely on grass pollen exposure. Even if having severe pollen allergy, participants will experience days with no or only mild symptoms alternating with days with severe symptoms as the pollen exposure varies[19]. In this trial, large day-to-day as well as year-to-year variation in the grass pollen counts were observed within each pollen region[20], causing corresponding variations in the level of symptoms and use of symptomatic medications. It has recently been suggested that data could be normalized for the peak 2 weeks of pollen season to adjust for seasonal and geographical variability in pollen counts[8]. However, this was not done for any of the efficacy analyses in the present trial.

There was a significant correlation between the RCS and the cumulative grass pollen counts in the beginning of the season, which highlights the significant dependency on pollen exposure to be able to measure efficacy in this type of trials. The correlation may relate to cumulated pollen counts as reported here, as well as intensity (i.e. maximum peak height) or onset pattern (i.e. how abrupt the onset of the pollen season is). Also the potency of the pollen grains, the release of lipid mediators and enhanced allergenicity caused by an interaction with air pollution[21-23] may influence the level of symptoms and thereby the efficacy measures.

Days with severe symptoms are by definition the most troublesome days where rhinoconjunctivitis symptoms exacerbate and significantly impact the participants' daily life; a fact that is highlighted by the correlation with the rhinoconjunctivitis quality of life scores. The significant decrease in days with severe symptoms and the improved quality of life in the grass AIT group both during the 3 treatment seasons and during the 2 follow-up seasons support the clinical relevance of the primary efficacy endpoints and emphasize that the effect of grass AIT treatment is relevant from the patient perspective.

The number of participants symptomatic to other allergens than grass was not systematically collected. Given the successful randomization on subject characteristics and the equal distribution of other sensitizations, it is likely that the distribution of poly-allergic subjects was equal between groups. The efficacy of grass AIT was similar in mono-and poly sensitized participants. There were no differences between groups in terms of new sensitizations. The preventive effect of immunotherapy suggested from other trials[24-27] relates to pediatric studies where the rate of new sensitizations is higher. It remains to be determined whether grass AIT may similarly reduce new sensitizations.

As a post hoc analysis, the asthma combined score was investigated. The significant effect of grass AIT on asthma supports the united airway disease hypothesis[28;29]. A trial is currently ongoing to assess if grass AIT can also reduce the risk of developing asthma in children[30].

Clinical efficacy following sublingual grass AIT treatment was associated with sustained increases in serum grass pollen allergen-specific IgG_4 antibodies, accompanied by parallel increases in serum IgE-blocking factor and in serum inhibitory activity for binding of allergen-IgE complexes to B cells. Importantly, the immunological changes were sustained 2 years after completion of treatment, like the observed clinical improvement, and entirely consistent with long-term disease-modification. The findings are furthermore in agreement with those observed after end of long-term subcutaneous immunotherapy[7], with the exception that after completion of grass AIT, the sustained increases in serum IgG_4 closely paralleled the increased serum blocking activity whereas after subcutaneous treatment, IgG_4 levels returned 80% towards baseline levels. This suggests that local regulatory mechanisms involving T-B cell interactions following sublingual application of allergen[31;32] may result in a greater proportion of IgG_4 with high avidity and hence ability to compete with IgE as compared to the subcutaneous route, where allergen is administered remote from the target organ, possibly resulting in B cells producing a broader range of both high and low avidity IgG_4, with only the 'functional' high avidity IgG_4 persisting long-term.

Conclusions

The sustained, significant and clinically relevant efficacy of the SQ-standardized grass AIT during 2 follow-up seasons and the parallel immunological changes confirms disease-modification; the one distinct feature separating specific immunotherapy from all other treatment options for allergy. No

safety issues were identified during the 3 years of treatment or the 2 follow-up years. Specifically, there were no events of anaphylaxis. The most common adverse events were mild to moderate local application site reactions that in most cases presented at treatment initiation.

In this trial, placebo participants were at all times more likely to have weeks containing days with severe symptoms during the pollen season compared to the grass AIT group. The number of days with severe symptoms was directly reflected in a worse quality of life. The significant decrease in days with severe symptoms and the improved quality of life in the active group support the clinical relevance of the primary efficacy endpoints and emphasize the relevance of grass AIT treatment from the patient perspective.

Thus, the grass AIT should be considered an important treatment option in patients with grass pollen induced rhinoconjunctivitis who remain uncontrolled on symptomatic medications.

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503 **Tables**

504 **Table 1:** Rhinoconjunctivitis symptom and medication scores during the 5 grass pollen seasons covered by the trial

	Original Trial	**Extension Trial**[†]			
	Season 1	**Season 2**	**Season 3**	**Follow-up season 4**	**Follow-up season 5**
N[*](Grass AIT)	282	172	160	142	137
N[*](Placebo)	286	144	127	115	104
Rhinoconjunctivitis Symptom Score					
Grass AIT: mean[¤] (median)	2.9 (2.6)	2.4 (1.9)	2.6 (2.0)	2.7 (2.3)	2.6 (2.2)
Placebo: mean (median)	4.1 (3.8)	3.8 (3.5)	3.6 (3.2)	3.6 (3.3)	3.4 (3.2)
Difference in means					
Absolute [CI $_{95\%}$]	1.3 [0.9; 1.7]	1.4 [0.9; 1.9]	1.0 [0.5; 1.6]	0.9 [0.4; 1.5]	0.8 [0.3; 1.4]
Relative to placebo	31%	36%	29%	26%	25%
p-value	<0.0001	<0.0001	<0.001	<0.001	0.004
Difference in medians					
Absolute	1.2	1.5	1.2	1.0	1.0
Relative to placebo	32%	44%	37%	31%	31%
Rhinoconjunctivitis Medication Score					

Grass AIT: mean (median)	1.7 (1.0)	1.7 (0.5)	1.8 (0.8)	2.3 (1.2)	2.4 (1.6)
Placebo: mean (median)	2.7 (2.2)	3.2 (1.7)	3.0 (2.1)	3.2 (2.6)	3.0 (2.1)
Difference in means					
Absolute [CI $_{95\%}$]	1.0 [0.6; 1.4]	1.5 [0.8; 2.2]	1.2 [0.5;1.9]	0.9 [0.1; 1.7]	0.6 [-0.1; 1.4]
Relative to placebo	38%	45%	40%	29%	20%
p-value	<0.0001	<0.0001	<0.001	0.022	0.114
Difference in medians					
Absolute	1.2	1.2	1.3	1.4	0.4
Relative to placebo	55%	73%	60%	52%	21%

*: numbers of participants in the analyses, i.e. all participants providing diary data during the grass pollen season without imputation of data; AIT: allergy immunotherapy tablet; ¤: all mean values refer to adjusted means; †: the trial was initially planned to cover a single grass pollen season, and when the trial was extended with another 4 years, 195 participants chose not to enroll or were not offered enrolment due to closure of sites. The participants in the extension were a representative subset of the population originally included in the trial (see Dahl et al. [11]).

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508 **Table 2:** Days with severe symptoms and RQLQ scores during the peaks of the 5 grass pollen seasons covered by the trial

Grass pollen seasons	**Percent days with severe symptoms, peak season**					**Mean RQLQ scores, peak season**			
	Placebo	**Grass AIT**	**Rel. diff. ***	**p-value**	**odds ratio[#]**	**Placebo**	**Grass AIT**	**Rel. diff.**	**p-value**
Season 1	17	8	52%	<0.0001	0.43	1.8	1.3	25%	<0.0001
Season 2	14	5	63%	<0.0001	0.34	1.4	1.0	29%	0.0003
Season 3	13	5	61%	<0.0001	0.35	1.4	1.0	32%	<0.0001
Follow-up season 1	16	8	49%	<0.0001	0.47	1.7	1.2	28%	0.0011
Follow-up season 2	13	6	54%	<0.0001	0.43	1.4	1.1	26%	0.0047

Days with severe symptoms are defined as a symptom score of 3 in any of the 6 rhinoconjunctivitis symptoms; the peak grass pollen season is defined as the 15 days period with the highest average pollen count; RQLQ: rhinoconjunctivitis quality of life questionnaire; *: Rel. diff.=((placebo-grass AIT)/placebo)×100%; [#]: for risk of having days with severe symptoms in the grass AIT group versus placebo

509 **Figure Legends**

510 **Figure 1:** Weighted rhinoconjunctivitis combined symptom and medication score for the 5 grass
511 pollen seasons of the trial and averaged over all seasons with relative differences between groups
512 and $CI_{95\%}$. All relative differences were statistically significant.

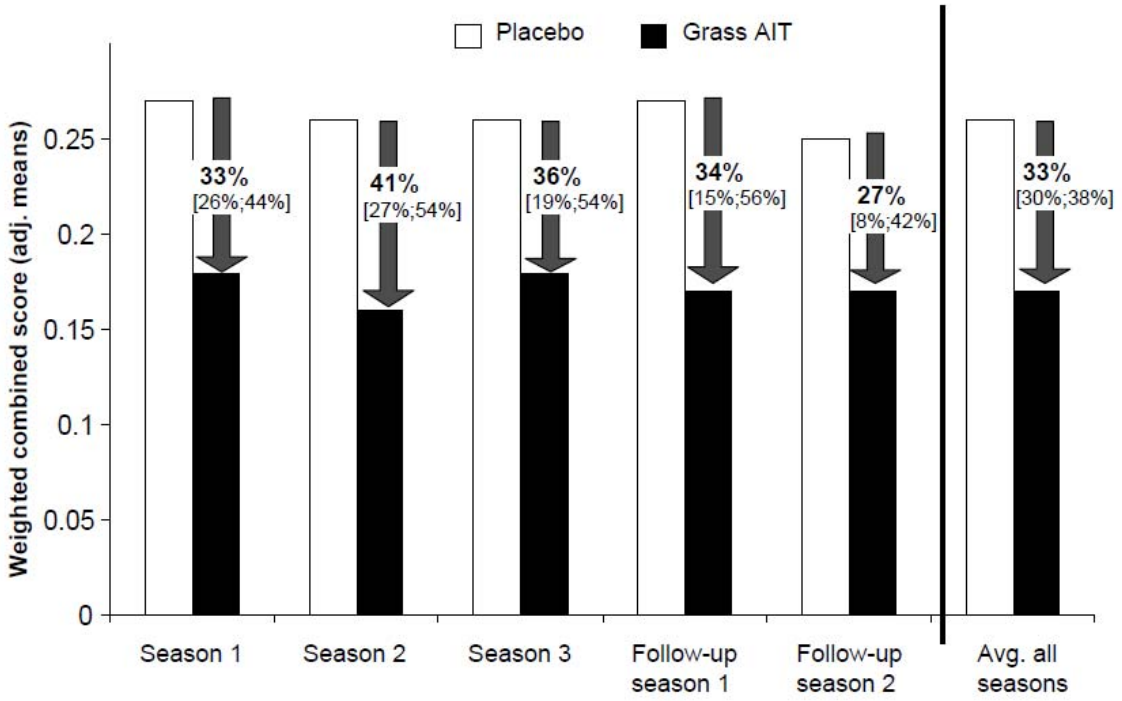

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Figure 2: Weighted rhinoconjunctivitis combined score for grass AIT and placebo as a function of grass pollen counts (left y-axis). The grey curve is the cumulated pollen curve (right y-axis). The plot is based on 80,958 diary data over the 5 years of the trial. The vertical bars are 95% prediction intervals.

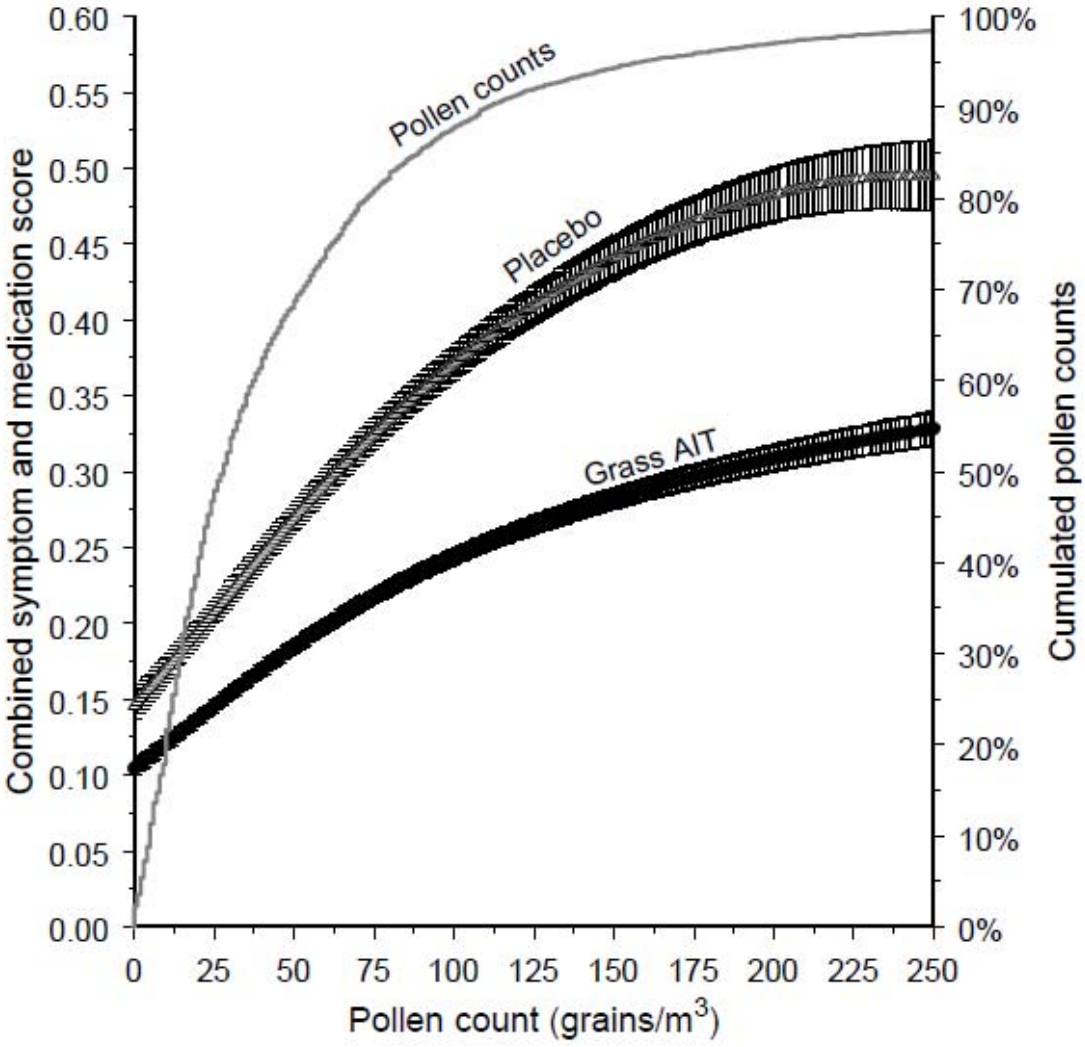
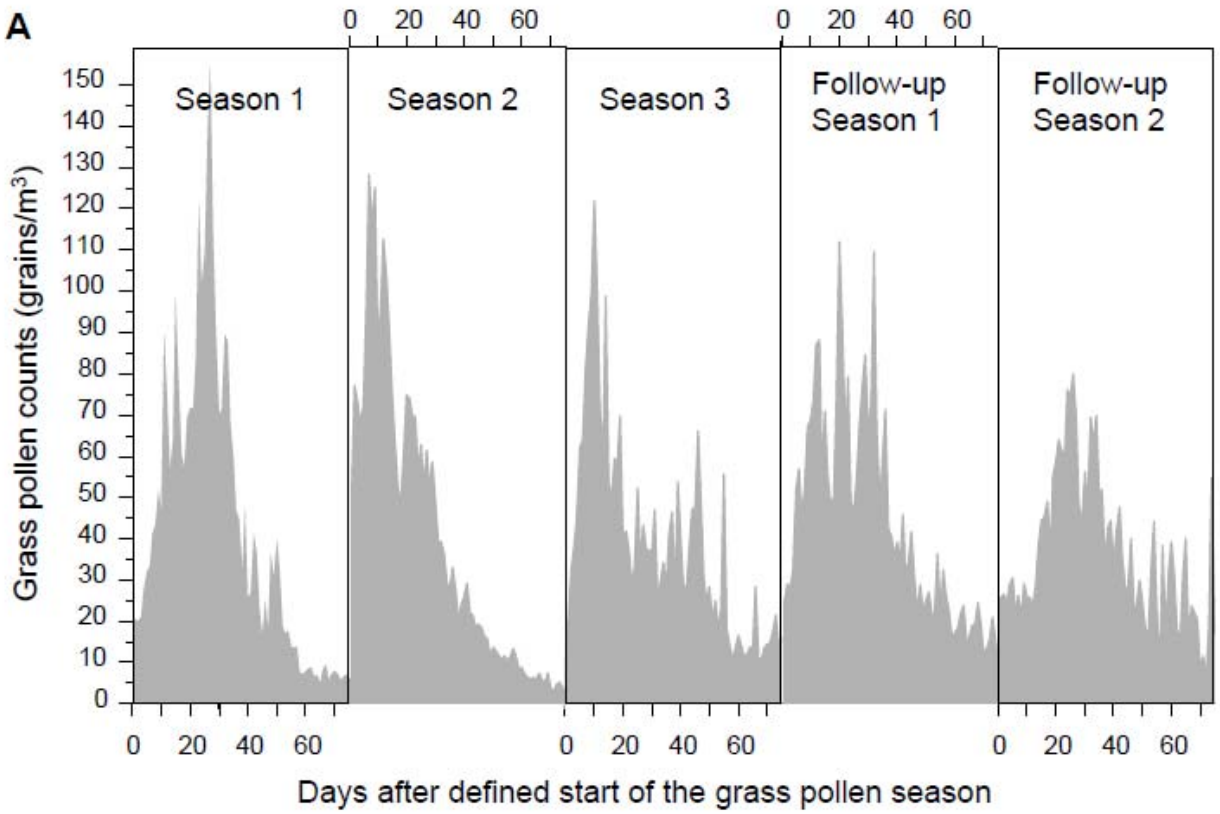
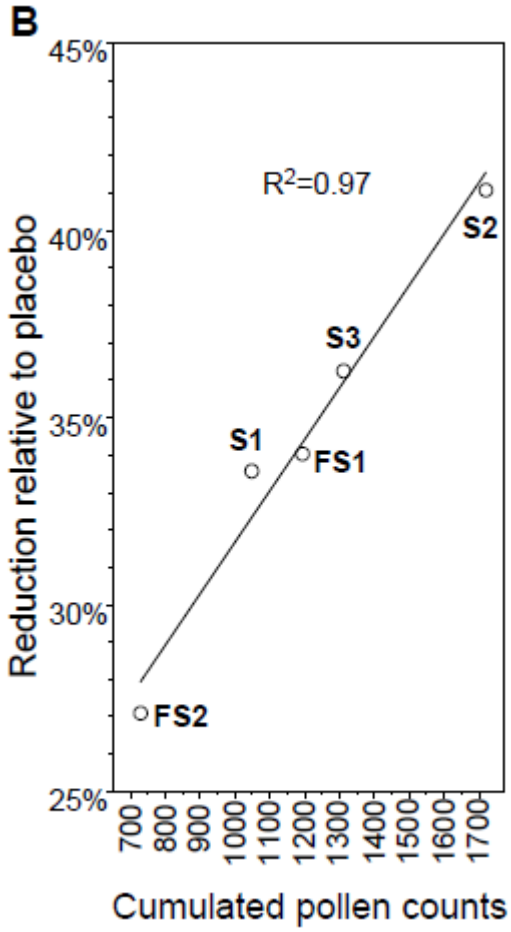


Figure 3: A: Average grass pollen exposures, for participants with diary data on the given day, during the 5 grass pollen seasons of the trial (2005-2009); B: Linear regression of the relative difference in the weighted rhinoconjunctivitis combined score against the cumulative grass pollen counts in the initial 3 weeks of the grass pollen season (S: season, FS: Follow-up season). Lowest cumulative counts were observed during the second follow-up season (FS2).




B
45%
40%
35%
30%
25%
Reduction relative to placebo
R2=0.97
S2
S3
S1
FS1
FS2
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
Cumulated pollen counts

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531 **Figure 4:** The relation between weekly overall rhinoconjunctivitis quality of life (RQLQ) score and
532 number of weekly days with severe symptoms (-Δ-, left axis) and the odds ratio for having days
533 with severe symptoms in the grass AIT group versus the placebo group (-●-, right axis). Data
534 included for all 5 seasons of the trial.

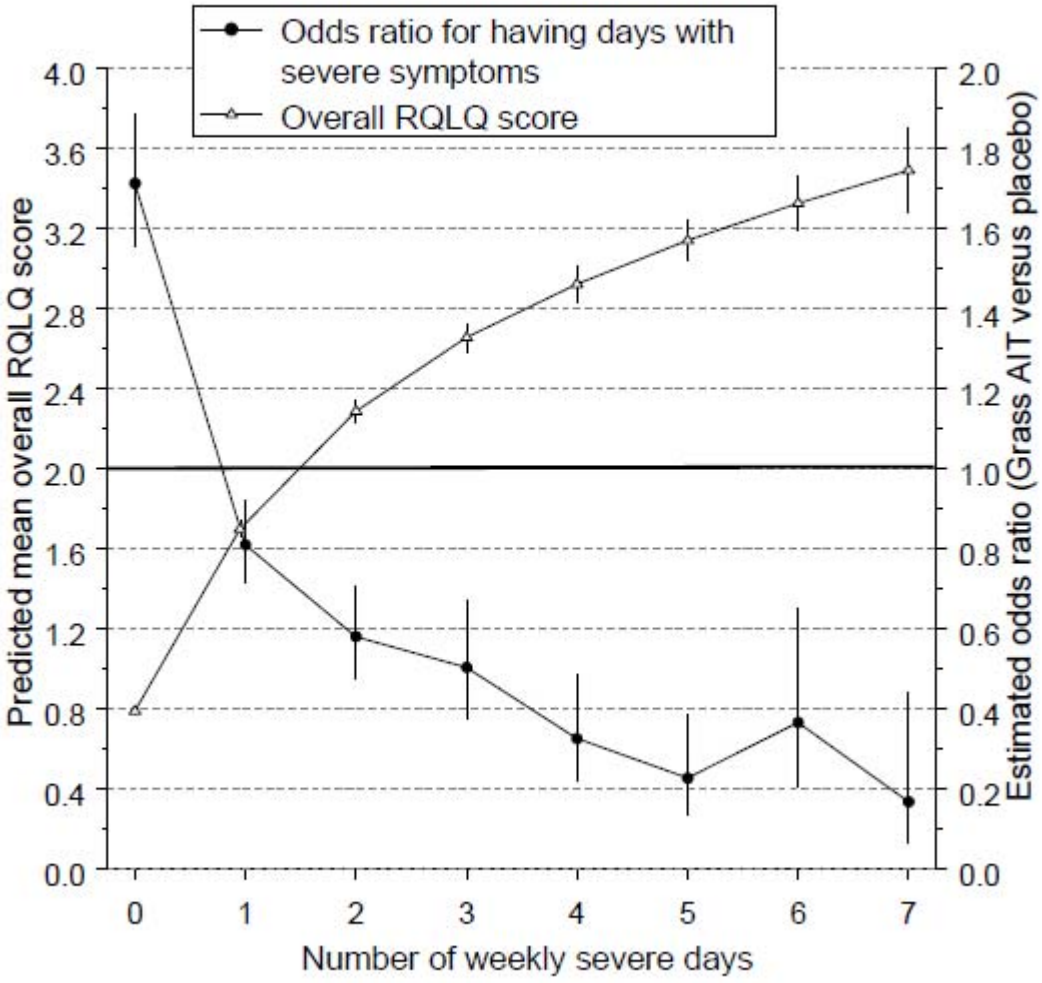

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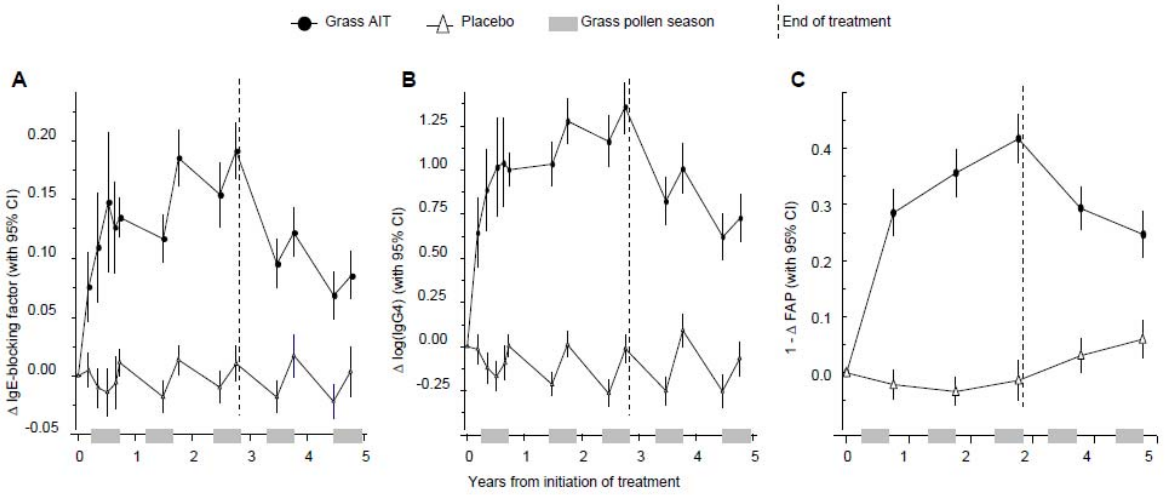
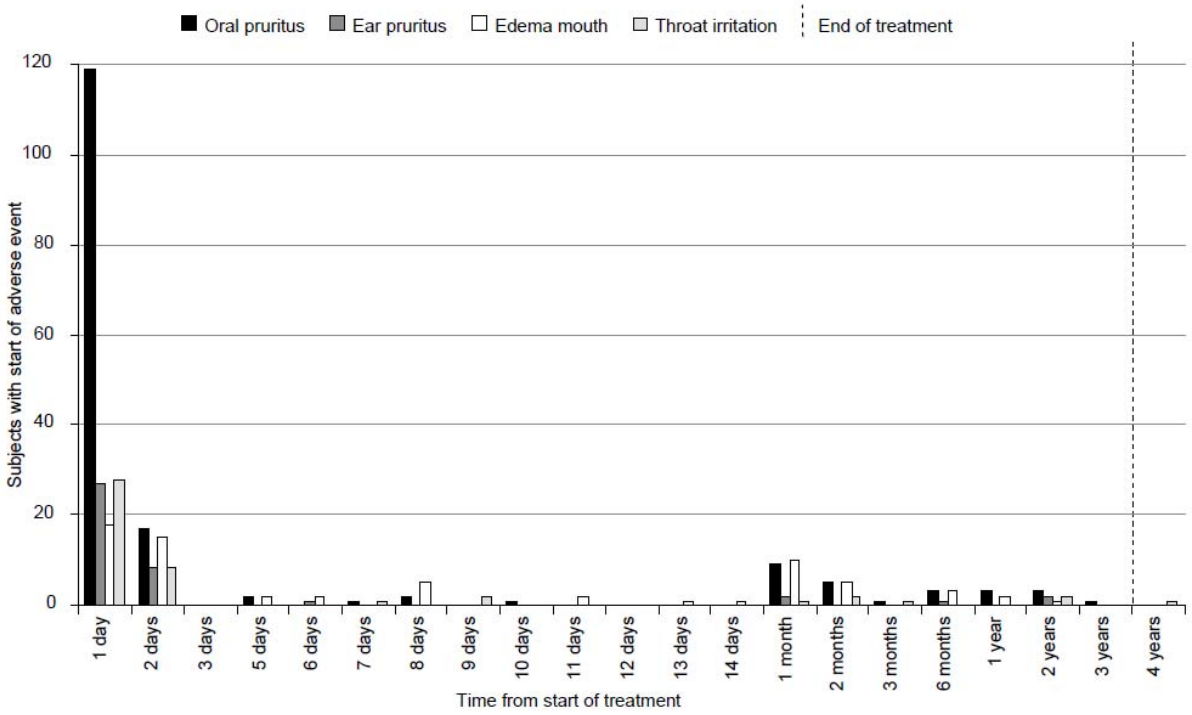
Figure 5: Change from baseline in specific IgE-blocking factor (A), specific IgG_4 (B) and FAP inhibition (C). Grass pollen seasons are indicated by grey boxes and end of treatment by dotted lines. The differences between grass AIT and placebo were significant at all assessments. All samples from years 1-5 were analyzed after the end of the trial. IgG_4 measurements for years 1-5 are presented using Phadia CAP RAST. In previous reports, IgG_4 analyses were performed using the Centaur system[33] for which reagents became unavailable for the last year of the trial.



545 **Figure 6:** Onset of the 4 most common types of adverse events (data from active group only). Note
546 that "1 day" refers to the same day as treatment was started. None of these types of adverse events
547 occurred during the 5th year of the trial.



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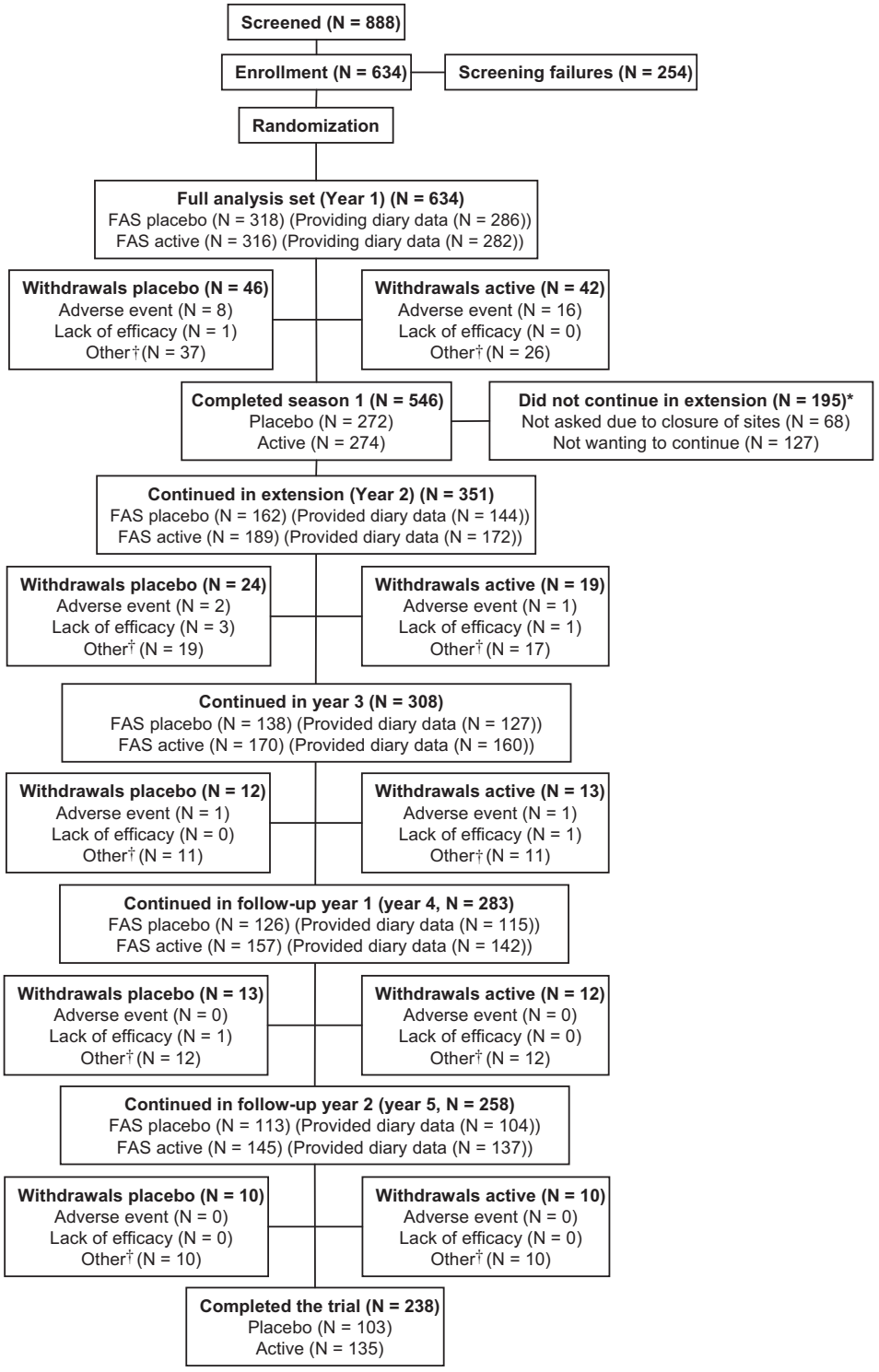

FIG E1. Overview of trial participants during the 5th year of the trial. *There were no statistically significant difference in treatment effect during the grass pollen season 2005 between participants continuing and participants not continuing in the extension. †Other reasons for withdrawal included "lost to follow-up," "pregnancy," "withdrawal of consent," "subject noncompliance," or "other."

TABLE E1. Rhinoconjunctivitis and asthma symptomatic medications score scheme

Symptomatic medications, maximum recommended daily dose	Score (dose)	Maximum score per day*
Rhinoconjunctivitis		
Desloratadine: 5 mg/tablet, 1 tablet once daily	6 (per tablet)	6
Olopatadine eye drops: 1.0 mg/mL, 1 drop per eye twice daily	1.5 (per drop)	6
Budesonide nasal spray: 32 μg/puff, 2 puffs per nostril twice daily	1 (per puff)	8
Prednisone: 5 mg/tablet, 10 tablets (50 mg) once daily	1.6 (per tablet)†	16
Maximum daily rhinoconjunctivitis medication score		36
Asthma		
Salbutamol: 200 μg inhalation, 2 inhalations twice daily	2 (per inhalation)	8
Fluticasone: 250 μg inhalation, 2 inhalations twice daily	2 (per inhalation)	8
Prednisone: 5 mg/tablet, 10 tablets (50 mg) once daily	1.6 (per tablet)†	16
Maximum daily asthma medication score		32

*If the recommended dose was exceeded, the actual score was used.
†Prednisone counted in the rhinoconjunctivitis score and/or in the asthma score depending on the symptoms.

TABLE E2. All adverse events leading to trial discontinuation during the 5-year trial period

No.	Preferred term	Treatment	Event start	Withdrawal	Relation*	Severity
Reported during 1st year of the trial						
1	Insomnia	Grass AIT	15-10-2004	16-11-2004	Possible	Mild
2	Edema mouth	Grass AIT	29-11-2004	05-01-2005	Probable	Moderate
	Oral pruritus		29-11-2004		Probable	Moderate
3	Throat irritation	Grass AIT	01-12-2004	26-01-2005	Probable	Moderate
4	Malaise	Grass AIT	03-12-2004	04-01-2005	Possible	Mild
5	Dysphonia	Grass AIT	10-12-2004	14-12-2004	Probable	Mild
	Oral pruritus		10-12-2004		Probable	Severe
	Pharyngeal edema		10-12-2004		Probable	Severe
6	Nausea	Grass AIT	12-12-2004	22-12-2004	Possible	Moderate
7	Bronchospasm	Grass AIT	12-12-2004	30-12-2004	Probable	Mild
8	Cough	Grass AIT	13-12-2004	13-12-2004	Probable	Moderate
	Dyspnea		13-12-2004		Probable	Moderate
	Edema mouth		13-12-2004		Probable	Moderate
	Pharyngeal edema		13-12-2004		Probable	Moderate
9	Tongue edema	Grass AIT	14-12-2004	21-12-2004	Probable	Mild
10	Localized edema	Grass AIT	15-12-2004	29-12-2004	Unlikely	Moderate
	Diarrhea		24-12-2004		Unlikely	Mild
11	Edema mouth	Grass AIT	23-12-2004	27-12-2004	Probable	Mild
12	Angioneurotic edema	Grass AIT	27-12-2004	30-12-2004	Probable	Moderate
13	Oropharyngeal swelling	Grass AIT	05-01-2005	07-01-2005	Probable	Moderate
14	Eye pruritus	Grass AIT	15-12-2004	11-01-2005	Probable	Moderate
	Oral pain		15-12-2004		Probable	Moderate
	Oropharyngeal swelling		15-12-2004		Probable	Moderate
15	Tongue edema	Grass AIT	11-01-2005	11-01-2005	Probable	Moderate
16	Vasculitis	Grass AIT	20-04-2005	03-05-2005	Possible	Mild
17	Pharyngeal edema	Placebo	22-11-2004	30-11-2004	Probable	Moderate
18	Headache	Placebo	07-01-2005	18-01-2005	Possible	Moderate
	Musculoskeletal stiffness		10-01-2005		Unlikely	Mild
19	Cesarean section†	Placebo	08-09-2005	17-01-2005	Unlikely	NA
20	Dyspnea	Placebo	30-03-2005	31-03-2005	Unlikely	Moderate
21	Subarachnoid hemorrhage‡	Placebo	04-04-2005	04-04-2005	Unlikely	Severe
22	Abdominal pain	Placebo	03-2005	25-04-2005	Unlikely	Mild
	Pharyngolaryngeal pain		03-2005		Possible	Mild
23	Brain neoplasm	Placebo	23-06-2005	28-06-2005	Unlikely	Severe
24	Nasal passage irritation	Placebo	12-07-2005	10-08-2005	Unlikely	Mild
Reported during 2nd year of the trial						
25	Arthritis	Grass AIT	12-2005	04-01-2006	Possible	Mild
26	Headache	Placebo	30-09-2005	01-12-2005	Possible	Mild
27	Viral infection	Placebo	03-04-2006	09-08-2006	Unlikely	Moderate
	Lymphadenopathy		31-05-2006		Unlikely	Moderate
Reported during 3rd year of the trial						
28	Asthma	Grass AIT	15-06-2007	09-08-2007	Probable	Moderate
29	Chlamydial infection (pneumonia)	Placebo	03-04-2007	24-04-2007	Unlikely	Moderate

*The investigators assessed whether the causality of the adverse event was probable, possible, or unlikely related to the investigational medicinal product.

†The participant discontinued because of pregnancy on January 17, 2005. On September 8, 2005, the participant was hospitalized and a cesarean section was performed because of fetal distress (umbilical cord was compressed). Mother and baby fully recovered and were discharged from hospital on September 15, 2005.

‡The participant died from the event.