

Original article

# Efficacy and safety of preseasonal-specific immunotherapy with an aluminium-adsorbed six-grass pollen allergoid

**Background:** The clinical efficacy and safety of a six-grass pollen allergoid has been studied. The advent of more exacting clinical guidelines and a better appreciation of the possible mechanisms of treatment prompted this reappraisal.

**Methods:** A 2-year double-blind multicentre placebo-controlled phase 3 clinical trial was undertaken in 154 patients suffering symptoms of rhinoconjunctivitis with or without asthma (GINA I or II). Therapy comprised two consecutive preseasonal short-courses of subcutaneous injections using a grass pollen allergoid adsorbed to aluminium hydroxide.

**Results:** A combined symptom and medication score (SMS) was used as the primary end-point for clinical efficacy. SMS from the first year showed a significant difference of 26.6% between the two study groups ( $P = 0.026$ ) and this was improved after the second year when there was a 48.4% difference in SMS between active and placebo treatment in favour of the allergoid ( $P = 0.018$ ). Highly significant increases in grass pollen allergen-specific IgG1 and IgG4 antibody concentrations were measured in association with active treatment. Allergen tolerance was increased as judged by a conjunctival provocation test and significant improvements in quality of life were documented using a standardized questionnaire. The allergoid was well tolerated.

**Conclusions:** The grass pollen allergoid was shown to be safe and clinically efficacious in the management of hay fever with or without asthma (GINA I or II).

**C. J. Corrigan<sup>1</sup> for the Study Group\*, J. Kettner<sup>2</sup>, C. Doemer<sup>2</sup>, O. Cromwell<sup>2</sup>, A. Narkus<sup>2</sup>**

<sup>1</sup>Guy's, King's and St Thomas's School of Medicine, London, UK; <sup>2</sup>Allergopharma Joachim Ganzer KG, Reinbek, Germany

\*The Study Group: Dr M. Henzgen, Jena; Dr W. Feußner, Kassel; Dr R. Dominicus, Dülmen; Dr Chr. Männer, Arnberg; Dr D. Stiller, Fürstenwalde; Dr S. Hofmann, Potsdam; Dr H. Scholz, Mahlow; Dr D. Futschik, Dresden; Dr C.J. Corrigan, London; Dr K. Rajakulasingam, London.

Key words: allergoid; grass pollen; hay fever; short-term immunotherapy; specific immunotherapy.

Dr C.J. Corrigan  
Department of Asthma  
Allergy and Respiratory Science  
Guy's, King's and St Thomas's School of Medicine  
Guy's Hospital  
London SE1 9RT  
UK

Accepted for publication 3 November 2004

Over the past decades there has been a substantial increase in the prevalence of IgE-mediated type I allergies in industrialized countries. Recent studies suggest that 25% of the European population suffers from allergies (1). Treatment with selective histamine H<sub>1</sub> antagonists and corticosteroids relieves allergy-related symptoms, but with the exception of allergen avoidance, specific immunotherapy (SIT) is the only treatment that can alter the natural course of allergic diseases (2). Traditional dosage regimens involved frequent injections, which were burdensome for patients and physicians, and the use of soluble antigen, which increased the risk of IgE-mediated adverse reactions. The introduction of depot preparations, adsorbed to aluminium hydroxide or other adjuvants, allowed the number of injections to be reduced, but only the development of chemically modified allergens (allergoids) achieved the combined goal of producing therapeutics with a reduced potential for causing side-effects and the maintenance of immunogenicity (3, 4).

Clinical studies have shown a good tolerance of aqueous grass and ragweed pollen allergoids in comparison with allergen preparations (5–9). Adsorption of pollen allergoids onto aluminium hydroxide suspensions results in depot preparations that have been shown to be well tolerated with good clinical efficacy in clinical studies (10, 11).

The study compound Allergovit<sup>®</sup> (Allergopharma KG, Reinbek, Germany), an aluminium hydroxide-adsorbed depot allergoid preparation of six-grass pollen allergens, was developed as a treatment for short-term preseasonal immunotherapy in pollinosis. In this study, the efficacy and safety of Allergovit<sup>®</sup> were investigated in a placebo-controlled design with preseasonal treatments during two consecutive years. For symptomatic relief the use of anti-allergic medication on demand was allowed in both treatment groups. Symptoms and use of anti-allergic medication were compared in the SIT vs the placebo group for the primary determination of efficacy using a symptom and medication score (SMS).

## Material and methods

### Patients

A total of 172 patients were selected in 10 centres according to the following criteria: (a) history of IgE-mediated, seasonal allergic rhinitis/conjunctivitis with or without asthma (GINA stage I and II) caused by grass pollen, (b) positive skin prick test result (wheal diameter >3 mm) to grass pollen extract, and (c) positive conjunctival provocation test with grass pollen allergens. Patients were excluded if they had undergone grass pollen immunotherapy in the previous 3 years, or suffered other seasonal allergies in the same period as the relevant grass pollen, or a history of cardiovascular or other immunological or medically relevant diseases.

### Study design

The study had a multi-centre, placebo-controlled design with pre-seasonal treatments during two consecutive years. A statistical interim analysis was performed after 1 year. Treatments comprised preseasonal weekly injections with either grass pollen allergoid (Allergovit®) or placebo until the expected onset of the grass pollen season, followed by an open, uncontrolled observational phase to provide adequate treatment with SIT over 3 years for all study patients. The double-blind phase was conducted between November 2001 and October 2003. The study design took account of the recommendations made by Malling (12) and was conducted in accordance with the guidelines for Good Clinical Practice and with the approval of local ethics committees. All patients gave written informed consent before admission to the study.

The study included a screening period of 2–3 months. Visits (V) were scheduled at screening (V-1 and V0), baseline (V1), treatment period (V2–V7), and grass pollen season (V8–V13) during the first year. For the second year, treatment started again in January 2003 (V1) following the same visit schedule as for the first year.

### Therapeutic preparations and treatment schedule

The investigational product was a preparation of extracts of six-grass pollen allergens (*Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*) treated with formaldehyde under controlled conditions to produce an allergoid, which was then co-precipitated with aluminium hydroxide. The resulting allergoid adsorbate was supplied as a suspension in two concentrations, strength A 1000 therapeutic units (TU)/ml and strength B (10 000 TU/ml), in physiological saline containing 0.4% phenol as preservative. A matching placebo solution supplied was used as comparator. All trial preparations were manufactured by Allergopharma Joachim Ganzer KG, Reinbek, Germany.

Subcutaneous injections commenced with 0.1 ml of strength A in January 2002 followed by an approximate doubling of the dose at intervals of 7–14 days up to 0.6 ml of strength B. The standard up-dosing regimen comprised of seven injections. Adjustments in the schedule were made according to individual tolerance. Once the maximum individually tolerated dose was reached, this was repeated after 2 weeks, and then every 4 weeks until onset of the grass pollen season.

### Symptom and medication score

All patients recorded daily symptoms and drug requirements on diary cards over periods of 12 weeks during the pollen season (from

15 May to 7 August). The primary end-point was the area-under-the-curve (AUC) of the daily sum of the SMS over a 42-day period. This period was defined for each centre on the basis of the regional pollen count, such that it started 10 days before and ended 31 days after the day with the highest pollen count. At least 75% of daily SMS scores had to be complete in order for a diary to be acceptable for evaluation. The severity of symptoms was assessed on a scale from 0 to 3 for nasal (sneezing, running, blocked), conjunctival (itching, tear flow, redness) and bronchial symptoms (cough, wheezing, asthma with dyspnoea), giving a possible maximum daily score of 27. Anti-allergic drugs in the form of topical alpha- or beta-mimetics, topical and oral antihistamines, topical nasal and inhaled corticosteroids, disodium cromoglycate (DSCG) eye drops, and short-term treatment with systemic corticosteroids were allowed for symptomatic relief of symptoms. Usage was rated according to the type, route and dose/number of applications to yield the medication score as follows (points per dose): topical antihistamines, DSCG, decongestants – 1; oral antihistamines – 6; nasal steroids – 3; short-acting bronchodilators – 1, prednisolone – 4 per 5 mg.

### Rhinoconjunctivitis quality of life questionnaire

A rhinoconjunctivitis quality of life questionnaire (RQLQ) (13) was included to support the primary efficacy criterion. Patients documented their 'quality of life' on diary cards before the first injection and every 2 weeks during the grass pollen season.

### Conjunctival provocation test

An aqueous solution of allergen (Allergopharma) was instilled in increasing concentrations from 0 to 5000 BU/ml (biological units as defined on the basis of equivalent skin test reaction to histamine) into alternate eyes (lower conjunctival sac) at 10-min intervals. The diluent solution served as a negative control. Immediate conjunctival sensitivity was recorded as the dose that induced a minimum of irritation/pruritus and redness/hyperaemia. Changes in tolerated grass pollen allergen concentrations from baseline visit to end of each grass pollen season were calculated for each patient.

### Visual rating scales

Patients assessed the severity of their allergic symptoms during the previous grass pollen season and the changes from baseline (last season before treatment 2001 compared with grass pollen season 2003). Ratings were performed retrospectively at the screening visit and at the end of each grass pollen season on a 10-point visual rating scale (VRS) (1 = very weak to 10 = very strong). Before the first injection and after the end of each grass pollen season, the patients' general well being was recorded on a 10-point scale with lower numbers reflecting a better overall well-being than higher numbers.

### Immunological changes in serum

Grass pollen-specific serum IgE, IgG1 and IgG4 antibody concentrations were measured immediately before and after each pre-seasonal treatment period. IgE antibodies were measured using the Allervance System (Allergopharma) in accordance with the manufacturer's instructions. IgG1 and IgG4 antibodies were measured by ELISA using grass pollen allergen-coated micro-titre plates and biotin-labelled subclass specific monoclonal antibodies (BD Biosciences, Heidelberg, Germany). Bound subclass antibodies were

detected using avidin-labelled alkaline phosphatase (Sigma-Aldrich, Taufkirchen, Germany) together with *para*-nitrophenyl phosphate (Sigma) as substrate.

### Pollen counts

Information on regional pollen counts for Germany was obtained from the 'Stiftung Deutscher Polleninformationsdienst' with pollen count stations in Erfurt, Göttingen/Lengler, Münster, Hagen, Potsdam, and Dresden. The corresponding information for two centres in the UK was supplied by the 'National Pollen Research Unit' with a pollen count station in London. Pollen counts of at least 20/m<sup>3</sup> occurred on at least 21 individual days.

### Statistics

Differences between the mean AUC of the SMS in both treatment groups were tested by a two-tailed Wilcoxon–Mann–Whitney *U*-test assuming an overall significance level of 5%. According to the adaptive design (14), an interim analysis was conducted at the end of the first year using the Wilcoxon–Mann–Whitney *U*-test at a two-sided significance level of 0.0183. The final analysis was based on the Fisher's combination criterion. The overall RQLQ changes from baseline to the next assessment following the highest pollen count were compared between the two treatment groups using the Wilcoxon–Mann–Whitney *U*-test. In general, summary statistics were used to describe the data. Incidences and the number of episodes were calculated for local adverse drug reactions, systemic adverse drug reactions and other adverse events.

### Results

Demographic and baseline characteristics showed no relevant differences between the two groups (Table 1). 25% of the active and 30% of the placebo patients had asthmatic symptoms at inclusion. Of 172 patients screened, 154 were randomized. Eleven (14%) patients in the active and 15 (20%) in the placebo group withdrew before the end of the placebo-controlled part of the study. Most of them were either lost to follow up or withdrawn due to violations of in/exclusion criteria.

All patients in the safety set received an average number of nine injections per study year. The individual maintenance dose for active or matching placebo treat-

ment, defined as the highest dose a patient received at least twice was similar in both groups. Median values of 6000 TU indicate that the majority of patients in the active group received the maximum study dose of 0.6 ml of strength B.

### Clinical efficacy: symptom and medication score

The difference between active and placebo treatment in the combined SMS described by the median AUC of the SMS increased from 26.6% after the first year to 48.4% after the second treatment period (Fig. 1). Both differences were statistically significant with  $P_1 = 0.0258$  for 2002 and  $P_2 = 0.0177$  for 2003 as shown in Table 2.

Analyses of the subgroups with and without asthma and comparisons of the AUC of SMS by pollen count ( $\leq 100/\text{m}^3$  and  $> 100/\text{m}^3$ ) and centre size ( $\leq 5$  patients and  $> 5$  patients) also showed consistently lower mean and median scores in the active treatment group. The largest decrease in the median AUC of the SMS was reached in the active group with asthma in the second treatment

Table 1. Demographic data (safety population,  $n = 154$ )

	Allergoid	Placebo
Number of patients	77	77
Sex		
Female	42	48
Male	35	29
Mean age in years (range)	35 (18–58)	34 (18–60)
No. of patients with asthma	19	23
Patients with symptoms of		
Rhinitis	77	77
Conjunctivitis	73	73
Results of skin prick testing*	9.1 ± 5.9	8.6 ± 4.5

\*Values represent the mean ± SD weal diameter to mixture of six grasses in mm.

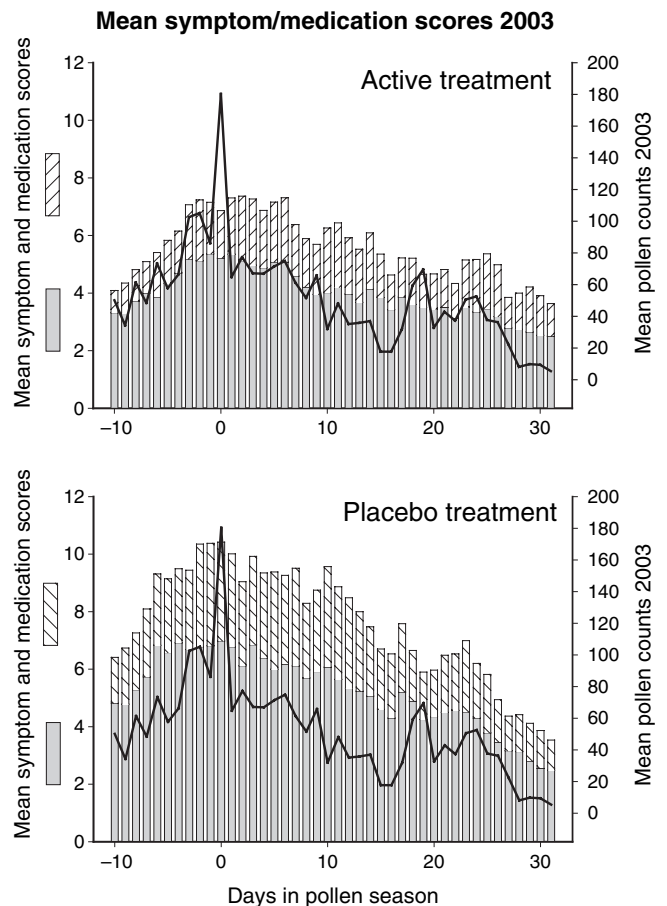


Figure 1. Combined symptom (filled bars) and medication (striped bars) scores (SMS) and grass pollen counts during the pollen season of the second treatment period (2003). Mean values. Full analysis set ( $n = 143$ ). Pollen counts – solid line.

Table 2. AUC of symptom and medication score (full analysis set,  $n = 143$ )

	Allergoid	Placebo	Allergoid vs placebo (%)	$P$ -value*
Total AUC of SMS 2002				
$n$	71	71		
Median	215	293	26.6	0.0258
Range	15.0–848.5	28.0–935.0		
Total AUC of SMS 2003				
$n$	66	60		
Median	174	337	48.4	0.0177
Range	0–663.5	11.0–866.0		

\*Two-tailed Wilcoxon–Mann–Whitney  $U$ -test.

year. Pollen count  $> 100/m^3$  generally resulted in higher median AUC of SMS values (active group vs placebo: 277 vs 329 in 2002 and 296 vs 356 in 2003).

Relative differences between the two treatments showed similar trends for symptoms and medication analysed separately. The analysis of symptoms revealed a difference of 31% between the active (median AUC 145) and placebo group (median AUC 210) after the second year. A difference of 69% was determined for the use of anti-allergic medication in the active (median AUC 17) as compared with the placebo group (median AUC 55). This indicates that a high-drug saving potential was realized in the actively treated group by SIT.

Quality of life and patients' individual ratings

A comparison of changes in quality of life from baseline to the peak of the grass pollen season between the treatment groups showed greater differences in the placebo group for all seven domains (activities, sleep, non-nose/-eye symptoms, practical problems, nasal symptoms, eye symptoms, emotional) (Fig. 2). The between-group difference in the median change in the overall

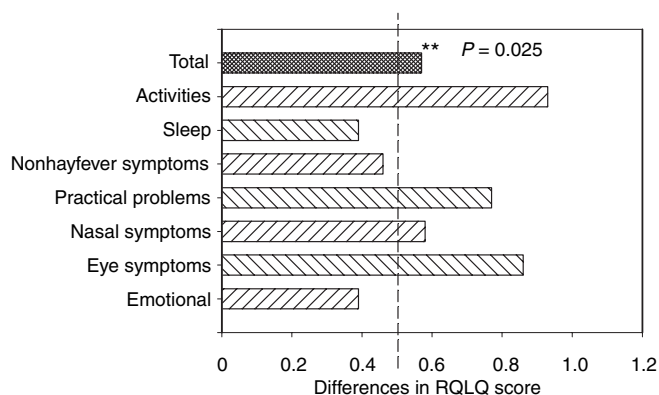


Figure 2. Impact of specific immunotherapy on quality of life. Figures show differences for active vs placebo group in changes in rhinoconjunctivitis quality of life (RQLQ) between before immunotherapy in 2002 and the grass pollen season 2003. Full analysis set ( $n = 143$ ). Dashed line corresponds to a difference of 0.5 in the RQLQ score which is regarded as clinically significant (18).

RQLQ [active group (-0.74) vs placebo group (-1.48)] was statistically significant ( $P = 0.0252$ ).

The patients' ratings of their general well-being reflected the negative impact of the grass pollen season. Consequently, both groups showed higher in-season values, indicating a worsening in their general condition at the end of the grass pollen season in 2003. However, the median change of 2.0 on the interval scale in the placebo group compared with a change of 1.0 in the active group showed an advantage in favour of the actively treated patients. Retrospective ratings on the severity of allergic symptoms during the last grass pollen season showed an improvement for both groups at the end of the second treatment year by an average of about 4 points on the VRS. Mean changes were slightly better for the active group, medians were identical.

Conjunctival provocation test

Compared with baseline, eight of 37 patients in the placebo-treated group (22%) showed a deterioration in allergen tolerance, 15 (41%) patients showed no change and 14 (38%) showed an improvement. In the active group 11 (26%) of 43 patients showed no change, whereas a large proportion, 31 of 43 (72%) patients in the active group improved their allergen tolerance (Fig. 3).

A comparison of the two treatment groups at the end of the grass pollen season in 2003 showed a significant difference ( $P < 0.0001$ , Wilcoxon–Mann–Whitney  $U$ -test) in the concentration of allergen tolerated.

Grass pollen-specific IgE, IgG1 and IgG4

The median serum concentration of allergen-specific IgE declined during the two treatment periods in both

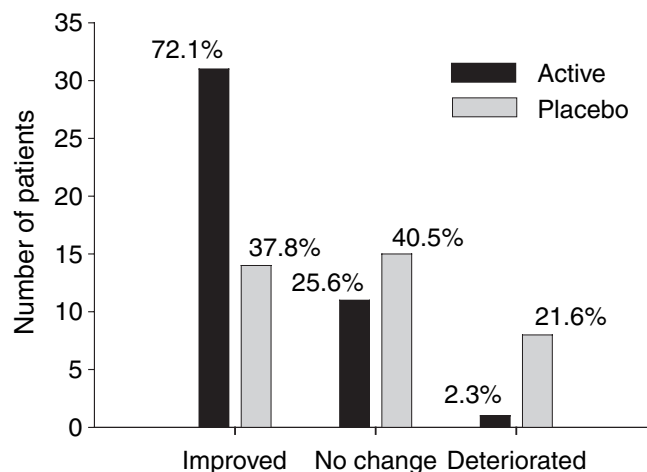


Figure 3. Changes in tolerated allergen concentration in conjunctival provocation testing comparing values before treatment in 2002 and after the second treatment period in 2003. Statistically significant difference between the groups ( $P < 0.0001$ ; Wilcoxon–Mann–Whitney  $U$ -test). Full analysis set ( $n = 143$ ).

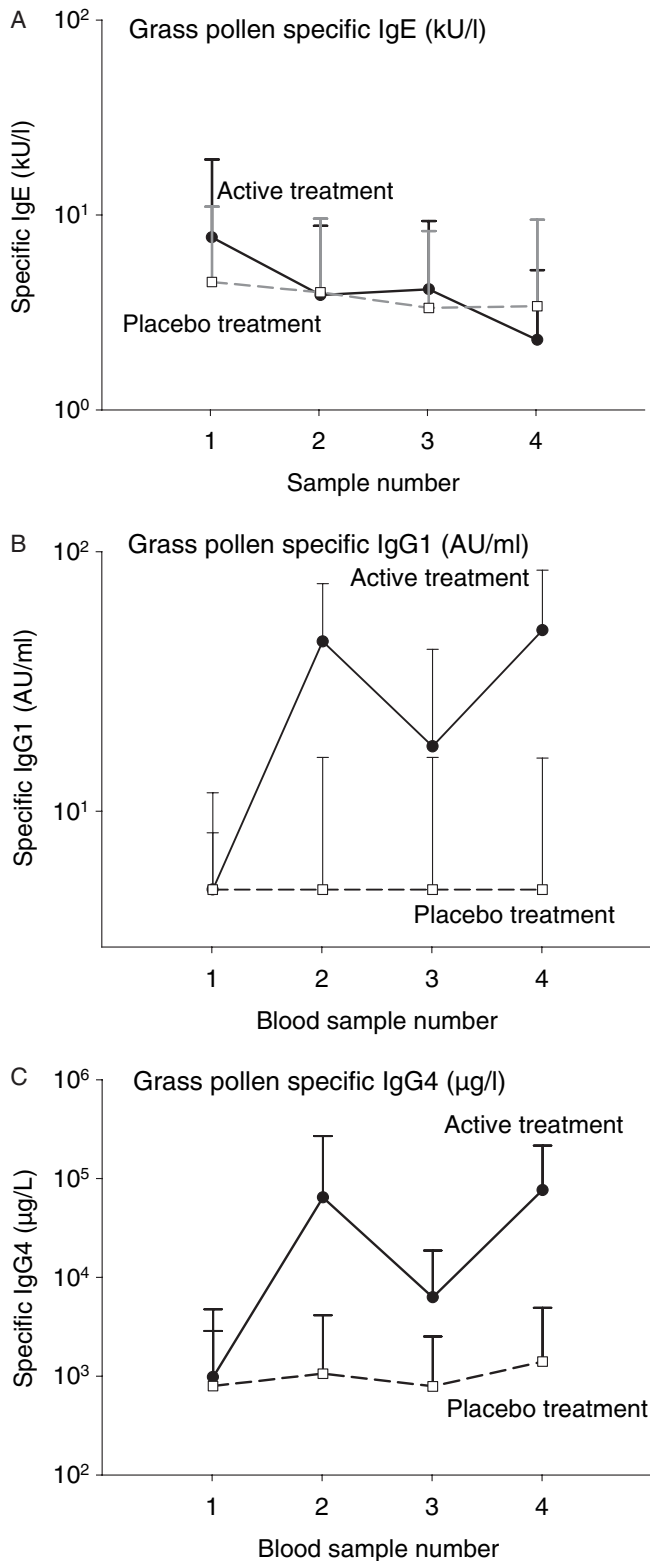


Figure 4. Changes in serum-specific IgE, IgG1 and IgG4 antibodies. Solid lines: allergoid, dotted lines: placebo treatment. Mean values  $\pm$  SD. Blood sample – 1: before immunotherapy, 2: grass pollen season 2002, 3: before immunotherapy 2003, 4: grass pollen season 2003.

treatment groups and over the study as a whole. The active preparation induced allergen-specific IgG1 antibody responses during the course of treatment in both 2002 and 2003, whereas no change was detectable in the placebo group. The median serum concentrations of allergen-specific IgG4 in the first study year increased 48-fold in those patients on active treatment with this response being boosted in the second year (when the level was increased 210-fold), clearly demonstrating the immunogenic activity of the active treatment. In the placebo group IgG4 concentrations increased very slightly at the end of the first (1.8-fold) and second pollen season (2.5-fold). Results for the whole treatment course are shown in Fig. 4.

#### Tolerability

The investigating physicians recorded all possible side-effects, including immediate and late reactions and systemic reactions as adverse events. The side-effects reported were those typically seen in association with subcutaneous allergen SIT, particularly local reactions, characterized by itching, transient local swelling and erythema at the injection site. Most events were of mild intensity and resolved quickly. Local reactions consistent with the allergic disposition of the patients were reported in 19.5% of actively treated patients. Five actively treated and two placebo-treated patients experienced late systemic reactions including urticaria, wheezing (in both groups) and itching of eyes. Unexpected reactions according to investigators (mainly large local swellings and itching) were reported in 10 actively treated and three placebo-treated patients. No serious drug-related adverse events occurred in the active treatment group and no immediate anaphylactic reactions were observed. The incidence of adverse events with probable, possible or definite relationship to study medication was comparable in the groups of patients with rhinitis/rhinoconjunctivitis and asthma. With regard to vital signs and laboratory parameters no special risks were identified.

#### Discussion

In this placebo-controlled study, preseasonal immunotherapy with an allergoid preparation of six-grass pollen allergens over 2 years was effective in reducing symptoms and requirements for drugs in patients with IgE-mediated, moderate to severe seasonal allergic rhinitis/rhinoconjunctivitis. Clinical improvement was also reflected in reduced conjunctival allergen sensitivity, improved quality of life and increases in grass pollen allergen-specific IgG antibody responses.

Allergic patients suffer from both the symptomatic manifestations of their disease and the need to use anti-allergic medication. The combined SMS as a measure of clinical efficacy takes account of both these factors.

Clinical efficacy was already evident after one preseasonal treatment cycle of an average of nine injections with the probability that benefit would continue with further treatment as shown in other studies (15) and in this study for the second treatment year. The placebo patients had higher symptom scores even when provided with anti-allergic medication. This result reflects a clinically relevant improvement for the active treatment group and exceeds a 30% difference compared with placebo suggested as a threshold for clinical efficacy in criteria for SIT drawn-up by Malling (16). In view of the fact that the study did not include the determination of a baseline SMS in the pollen season preceding immunotherapy, the relative improvements in SMS can be viewed as even more impressive. The decrease in the SMS seen in the subgroup with asthma, particularly in the second treatment year, is concordant with the effects of SIT in reducing bronchoconstrictor responses to allergen challenge (17).

This study demonstrates the suitability of the RQLQ as an instrument for measuring efficacy of SIT. The overall score, as well as the seven domains of the RQLQ, seem to be useful candidates for the determination of treatment effects observed with preseasonal SIT. As the overall between-group difference exceeded the threshold value of 0.5 for minimal important differences in RQLQ elaborated by Juniper et al. (18), they also represent a clinically relevant effect of SIT on the rhinitis-specific quality of life. Therefore, although SIT with the depot allergoid preparation may not cure the allergic disease, it decreases the severity of symptoms and has the potential to increase quality of life.

The increased allergen tolerance, as demonstrated by the conjunctival provocation test results, substantiates the SMS and RQLQ findings. The conjunctival provocation test has been shown to be an effective measure of allergen tolerance even in those patients without conjunctival symptoms in addition to those of rhinitis (19), and produces results concordant with nasal provocation. The conjunctival test is easier to perform and lends itself better to multicentre studies, as it is easier to reproduce between centres. The results of the study confirm previous findings on safety and efficacy of Allergovit® (20, 21) where a clear improvement in SMSs was achieved after just 1 year of treatment in patients allergic to grass pollen, including asthmatics. Immunotherapy also beneficially influences the antibody titres for specific IgE, IgG1 and IgG4. The magnitudes of the IgG1 and IgG4 grass

pollen allergen-specific antibody responses clearly demonstrate the immunogenic activity of the allergoid preparation. The noninflammatory IgG4 antibodies may play a role as blocking antibodies by inhibiting IgE–allergen interactions that would lead to mast cell and basophil activation and IgE-mediated allergen uptake by antigen presenting cells, and thus preventing the subsequent enhancement of Th2 responses and the allergic phenotype (22, 23).

It has been noted that the use of depot or chemically modified allergen extracts may substantially improve the benefit : risk ratio of SIT by degrading IgE-binding epitopes, while retaining the antigenic properties relevant to desensitization (24). This view is supported by data from a survey of spontaneous safety reports for Allergovit® that showed that every 7500th patient has a risk of developing a serious systemic reaction as opposed to every 5000th patient treated with a comparable unmodified depot-preparation (25). The necessity for fewer injections, which can be administered over relatively short periods before the pollen season, provides a basis for enhanced compliance, and this is also cost-effective in comparison with purely symptomatic treatment. A long-term efficacy study of preseasonal SIT in children (26) has shown a prolonged clinical benefit in comparison with pharmacological treatment alone as well as reduction of new sensitizations.

The present study provides clear evidence for the clinical efficacy of the grass pollen allergoid preparation. It confirms the validity of the concept of using hypoallergenic allergen derivatives, in this case chemically modified grass pollen extract, and demonstrates that short-term preseasonal immunotherapy in pollinosis is effective.

### Acknowledgments

The study was conducted in 10 centres and made possible by the commitment and dedication of the following clinical investigators: M. Henzgen, Jena; W. Feußner, Kassel; R. Dominicus, Dülmen; Chr. Männer, Arnsberg; D. Stiller, Fürstenwalde; S. Hofmann, Potsdam; H. Scholz, Mahlow; D. Futschik, Dresden; C.J. Corrigan, London; K. Rajakulasingam, London. Allergen-specific antibody determinations were undertaken by Professor H. Fiebig and Dr B. Weber.

AMS, Mannheim, Germany was responsible for much of the study organization as well as the statistical analysis. This study was sponsored by Allergopharma Joachim Ganzer KG, Reinbek, Germany.

### References

1. de Monchy J, Andersen PS, Bergmann KC, Chivato T, Holm-Hansen A, Jarisch R et al. Living and learning with allergy: a European perception study on respiratory allergic disorders. *Respir Med* 2004;**98**:404–412.
2. Bousquet J, Lockey R, Malling H. WHO Position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1998;**53**(Suppl.):44.
3. Maasch HJ, Marsh DG. Standardized extracts: modified allergens – allergoids. *Clin Rev Allergy* 1987;**5**:89–106.

4. Akdis CA, Blaser K. Regulation of specific immune responses by chemical and structural modifications of allergens. *Int Arch Allergy Immunol* 2000;**121**: 261–269.
5. Norman PS, Lichtenstein LM, Marsch DG. Studies of allergoids from naturally occurring allergens. IV. Efficacy and safety of long-term allergoids treatment of ragweed hay fever. *J Allergy Clin Immunol* 1981;**68**:460–470.
6. Norman PS, Lichtenstein LM, Kagey-Sobotka A, Marsch D. Controlled evaluation of allergoids in the immunotherapy of ragweed hay fever. *J Allergy Clin Immunol* 1982;**70**:248–260.
7. Bousquet J, Hejjaoui A, Skassa-Brociek W, Guérin B, Maasch HJ, Dhivert H et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. *J Allergy Clin Immunol* 1987;**80**:591–598.
8. Bousquet J, Frank E, Soussana M, Hejjaoui A, Maasch HJ, Michel FB. Double-blind, placebo-controlled immunotherapy with a high-molecular-weight, formalinized allergoid in grass pollen allergy. *Int Arch Allergy Appl Immunol* 1987;**82**:550–552.
9. Bousquet J, Hejjaoui A, Soussana M, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. IV. Comparison of the safety and efficacy of two dosages of a high-molecular-weight allergoid. *J Allergy Clin Immunol* 1990;**85**: 490–497.
10. Pastorello EA, Pravettoni V, Incorvaia C, Mambretti M, Franck E, Wahl R et al. Clinical and immunological effects of immunotherapy with alum-adsorbed grass allergoids in grass-pollen-induced hay fever. *Allergy* 1992;**47**:281–290.
11. Tari MG, Mancino M, Ghezzi E, Frank E, Cromwell O. Immunotherapy with an alum-adsorbed Parietaria-pollen allergoid: a 2-year double-blind placebo-controlled study. *Allergy* 1997;**52**:65–74.
12. Malling HJ. Methodology and quality of immunotherapy trials. *Allergy* 2004;**59**:482–484.
13. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. *Clin Exp Allergy* 1991;**21**:77–83.
14. Bauer P, Koehne K. Evaluation of experiments with adaptive interim analysis. *Biometrics* 1994;**50**:1029–1041.
15. Mosbech H, Osterballe O. Does the effect of immunotherapy last after termination of treatment? Follow-up study in patients with grass pollen rhinitis. *Allergy* 1988;**43**:523–529.
16. Malling HJ. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998;**53**:461–472.
17. Arvidsson MB, Lowhagen O, Rak S. Allergen specific immunotherapy attenuates early and late phase reactions in lower airways of birch pollen asthmatic patients; a double blind placebo-controlled study. *Allergy* 2004;**59**:74–80.
18. Juniper EF, Thompson AK, Ferrie PJ, Roberts JN. Development and validation of the mini rhinoconjunctivitis quality of life questionnaire. *Clin Exp Allergy* 2000;**30**:132–140.
19. Riechelmann H, Epple B, Gropper G. Comparison of conjunctival and nasal provocation test in allergic rhinitis to house dust mite. *Int Arch Allergy Immunol* 2003;**130**:51–59.
20. Frank E, Williams A, Cromwell O, Atkinson P, Rajakulasingam K. Effectiveness of a pre-seasonal allergoid immunotherapy in patients with seasonal allergic rhinitis due to grass pollen. *J Allergy Clin Immunol* 2001;**107**: S260.
21. Williams AM, Frank E, Meyer H, Cromwell O, Rajakulasingam RK. Effectiveness of pre-seasonal allergoid immunotherapy in patients with grass pollen allergy. *Allergy* 2002;**57**(Suppl. 73):51–52.
22. Wachholz PA, Durham S. Induction of 'blocking' IgG antibodies during immunotherapy. *Clin Exp Allergy* 2003;**33**:1171–1174.
23. Wachholz PA, Kristensen Soni N, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;**112**: 915–922.
24. Anonymous. Position paper on allergen immunotherapy. Report of a BSACI working party. *Clin Exp Allergy* 1993;**23**(Suppl. 3):27–31.
25. Thum-Oltmer S, Jäger L. Specific immunotherapy with allergoids: effective, safe, and long-lasting. *Mod Aspects Immunobiol* (in press).
26. Eng PA, Reinhold M, Gnehm HPE. Long-term efficacy of pre-seasonal grass pollen immunotherapy in children. *Allergy* 2002;**57**:306–312.