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# Early improvement of patients' condition during allergen-specific subcutaneous immunotherapy with a high-dose hypoallergenic 6-grass pollen preparation

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## KEY WORDS

*Subcutaneous immunotherapy (SCIT), high-dose hypoallergenic preparation (allergoid), grass pollen allergy, short-term SCIT*

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## SUMMARY

**Objective:** *A double-blind, placebo-controlled study was performed with 37 patients to assess the efficacy as well as the safety of short-term pre-seasonal subcutaneous immunotherapy (SCIT) with a six-grass pollen allergoid for one year. Results:* After one pre-seasonal treatment cycle there are significant differences between the groups in favour of active treatment. On a "1 – 10" visual rating scale patients' condition is improved in the active group by a median of 2.5 points, whereas no change is found in the placebo treated group ( $p=0.024$ ). Thirteen of 20 (65%) actively treated patients in comparison to 6 of 17 (35%) placebo treated patients improved for at least two points which was defined as clinically relevant. The efficacy of SCIT is further demonstrated by significantly reduced skin test reactivity and significant increases in immunological parameters like allergen-specific IgG1 and IgG4 in actively treated patients. Injections are well tolerated and only mild to moderate systemic reactions occurred. **Conclusion:** *The high-dose hypoallergenic grass pollen preparation was shown to be safe and clinically efficacious. One pre-seasonal course of 7 SCIT injections was sufficient to reach significant and clinically relevant efficacy with good safety.*

## Introduction

Allergic rhinitis has a higher prevalence rate in Europe and is a significant health problem because of the high burden of uncontrolled symptoms which have impact on sleep, general well-being and health-related quality of life among patients (1, 2). A number of studies stress the importance of allergen-specific immunotherapy (SIT) as the only causal treatment when allergen avoidance is not possible. Subcutaneous immunotherapy (SCIT) provides both clinical and immunological tolerance with long-term efficacy and prevention of progression in allergic diseases

(3-5). Since safety and patients' compliance are of great importance, significant amount of work has been undertaken towards more 'convenient' and safer SCIT preparations for short-term application. Allergoids are one form of preparations produced by allergens being submitted to chemical and physical modifications such as aluminium-adsorption. Allergoids therefore have reduced allergenicity, yet maintain excellent immunogenicity (6). Allergovit® is one of the commercially available allergoid preparations on the European market whose efficacy and safety has been proven by various clinical trials (7-11). To extend the knowledge on efficacy, tolerance and mechanisms of SC-

IT, clinical studies are continuously conducted to supply further data. The present double-blind, placebo-controlled trial was performed as a mono-centric investigation. The purpose of the trial was to investigate efficacy and safety during the first double-blind, placebo-controlled treatment year after only 7 pre-seasonal injections in an Intention-to-treat (ITT) population.

## Material and methods

### Study design

The study was performed in a randomized, double-blind, placebo-controlled design for one year. All patients received active treatment in the following two years to achieve the recommended active treatment phase of 2 to 3 years. The trial was conducted in accordance with guidelines for Good Clinical Practice and was approved by the local Ethics Committee. All patients gave their written informed consent.

### Patients

A total of 38 adult subjects were recruited for the study whose demographic data are shown in table 1. All of them had clinical symptoms of IgE-mediated rhinitis/rhinoconjunctivitis with or without asthma (GINA I, II) attributed to grass pollen. Additional inclusion criteria were: positive skin prick testing to a grass pollen extract with a

wheel at least as large as positive control reaction (histamine dihydrochloride 1 mg/ml). Exclusion criteria were in accordance with the EAACI position paper (4), e.g. grass pollen immunotherapy in the previous three years, symptoms or strong skin test reactivity to other allergens occurring during the grass pollen season or perennial allergens, intake of  $\beta$ -blockers or ACE-inhibitors, FEV<sub>1</sub><75% predicted, cardiovascular and immunological diseases.

Thirty-seven patients received at least one injection: 20 patients active treatment and 17 patients matched placebo. Data of this intention-to-treat (ITT) population was considered for the evaluation of visual rating scale (VRS) as the primary efficacy endpoint.

Additionally, these patients were also included in the Safety Set for safety evaluation.

During a blinded review, it became obvious that there were some problems performing nasal provocation testing with no clear positive results in 3 patients. But patients' history and skin test results indicated a grass pollen allergy. This discrepancy between skin and provocation testing is noticed in many studies and may be related to the heterogeneity of mast cells and basophilic cells between the skin and the mucosa (12, 13). In the daily practice patients' history and skin (prick) test results are the cornerstones of allergy diagnosis and nasal provocation testing is generally performed only when discrepancies arise or difficulties exist in the assessment of patient's medical history and the results of skin and/or serological tests (14). Therefore, it was decided to include these patients in the ITT population to reflect real life

**Table 1** - Demographic data

	Active (n=20)	Placebo (n=18)
Age, mean (range)	33.45 (23-54)	32.39 (22-49)
Sex, male/female	8/12	8/10
% of patients with positive skin prick test results to		
Grass pollen only (monosensitized)	22%	53%
Grass pollen and other allergens* (polysensitized)	78%	47%
% of patients with nose symptoms	100%	100%
Thereof with daily intake of medication	60%	78%
% of patients with eye symptoms	100%	89%
Thereof with daily intake of medication	67%	69%
% of patients with lung symptoms	70%	61%
Thereof with daily intake of medication	7%	27%

\* out of a panel of early- and late blooming tree pollen mixtures, *Artemisia vulgaris*, cat, dog, house dust mite *Dermatophagoides pteronyssinus*, moulds

setting in the daily practice. In contrast, these patients and 4 others who had received at most 4 injections due to an unexpected early start of the grass pollen season were excluded from the per-protocol set (PP) (17 active, 13 placebo) for evaluation of secondary efficacy parameters (Fig. 1).

#### *Trial preparations, treatment and safety recommendations*

The trial medications were manufactured by Allergopharma Joachim Ganzer KG, Reinbek, Germany, according to GMP-Guidelines and supplied for blinded use: a) aluminium hydroxide-adsorbed allergoid (Allergovit®) of 6-grass pollen mixture (*Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*) and b) matching placebo suspension of physiological saline with aluminium hydroxide, caramel as a colouring agent and 0.0125 mg/mL (strength A) or 0.125 mg/mL (strength B) histamine dihydrochloride for blinding.

Treatment was performed with increasing doses of strength A (1,000 TU) (0.1; 0.2; 0.4; 0.8 ml) and strength B (10,000 TU containing 42 µg grasses group 5/ml) (0.15; 0.30; 0.60 ml). Injections were given subcutaneously at weekly intervals provided that the previous dose was tolerated well; otherwise dose modifications were recommended. The maximum dose of 0.6 ml of strength B was not to be exceeded and treatment was discontinued prior to the onset of the pollen season.

Following each injection the patient was kept under close supervision for at least 60 minutes and the patient's condition was assessed before being discharged. Side effects were recorded and classified according to the localization (local or

systemic), and the time of appearance (immediate or delayed). The following dosage modifications were performed in case of adverse reactions: local reactions with a diameter of 5-10 cm - the earlier dose was repeated; local reaction with a diameter of > 10 cm - the last well tolerated dose was repeated. Following a mild systemic reaction the dose was reduced by 2-3 steps and the therapy was restarted using strength A for those subjects who had severe systemic reactions.

#### *Clinical and immunologic parameters*

The primary end point of the study was the group difference of changes in patients' condition between baseline and in the grass pollen season after one pre-seasonal treatment course measured by a 10-point visual rating scale (with 1 point = very good; 10 points = very poor). Due to the recommendations by the authorities the primary endpoint had to be evaluated in terms of "success" and "non-success". It was accepted that a reduction of two or more points as significant and "success" when values were used for an individual treatment assessment. This is in accordance with another recent SCIT-trial (15).

Patients' assessment of quality of life was conducted after each year of pre-seasonal SCIT and was based on the question "Were there changes in your quality of life concerning your allergy?" The patients answered on an eleven point scale from '+5' increased to '-5' decreased with '0' indicating no change.

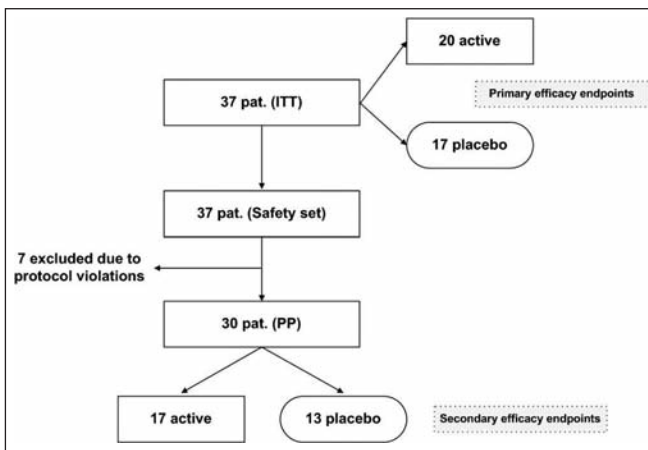
The physicians' blinded assessment of changes of patients' condition and need of anti-allergic medication was based on the questions if there was an improvement, no change or deterioration and a decrease, no change or an increase respectively.

Moreover, wheal areas of the quantitative skin prick test were compared before therapy and after therapy but before the onset of the pollen season. Patients were tested with a negative control solution (NaCl) twice, a positive control histamine dihydrochloride (0.1% and 1.0%) and with five 5-fold increasing concentrations of grass pollen extract, while each concentration was repeated three times. Then the threshold concentration was evaluated which was defined as the lowest allergen concentration at which the mean wheal size of the grass pollen extract was greater than that of histamine dihydrochloride 1%.

Nasal provocation testing with five increasing doses of grass pollen extract was performed before start of therapy and after 12 months.

Serum was taken from the patients before and during therapy and at the end of the study and changes of the

**Figure 1** - Flow chart during the double-blind, placebo-controlled trial phase. ITT indicates intention-to-treat, PP per-protocol set.



grass pollen-specific IgE-, IgG1- and IgG4-antibodies were assayed.

### Pollen counts

The grass pollen season was defined as the seven weeks with the strongest pollen count in London. This consisted of the week with the highest pollen count, two weeks before and four weeks after. Pollen count data were provided by the National Pollen Research Unit in Great Britain.

### Statistics

Based on previous data with a mean difference of 1.9 VRS - points (common standard deviation: 2.1) a sample size of 18 and 18 for the two groups was proposed to achieve a power of at least 75% for the two-sided t-test with an alpha evaluation of 5%. The comparability of the two groups was verified, and the effects of treatment were compared by two-sided Wilcoxon-Mann-Whitney U-Test in respect of differences between the groups (last observation carried forward (LOCF) in case of missing data after one pre-seasonal treatment course). Changes within the groups were verified by the Wilcoxon-signed rank test and Fisher's exact test for binominal distribution. Statistical analysis was performed using SPSS 8.0 and later versions. The overall significance level was 5%.

Figure 3 was prepared using SigmaPlot 11.0 (SYSTAT Software GmbH, Erkrath, Germany).

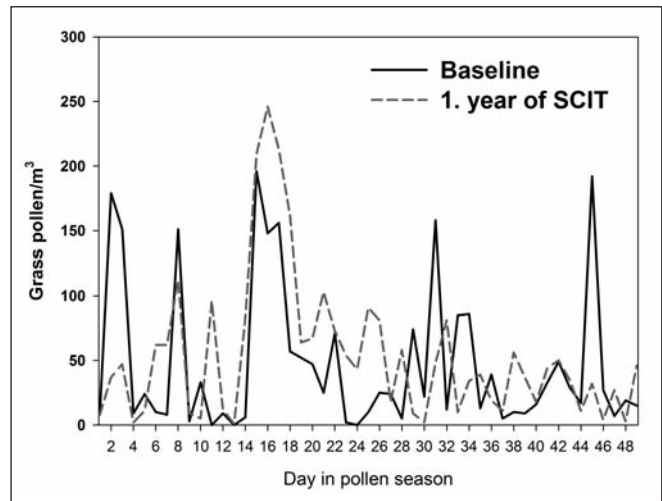
## Results

The grass pollen evaluation periods lasted from June, 5 to July, 23 in the baseline season and from May, 30 to July, 17 in the treatment year. Grass pollen counts were comparable at baseline and after one pre-seasonal course of SCIT (Fig. 2). 20 subjects were treated with the active preparation while 17 patients received placebo. Patients in both groups received at most 7 injections, only 1 patients of the active group received 8 injections. The cumulative dose of grasses group 5 allergen was 48 µg after 7 pre-seasonal injections applied with the dosage scheme mentioned above.

### Clinical efficacy after one treatment year

In the pollen season prior to treatment, VRS was available for 20 actively treated patients (mean, SD 7.2 ±1.8) and 17 placebo patients (mean, SD 7.0 ±1.3) without signifi-

**Figure 2** - Grass pollen count during the evaluation periods at baseline and after the pre-seasonal treatment course with Allergovit® Grasses or placebo.

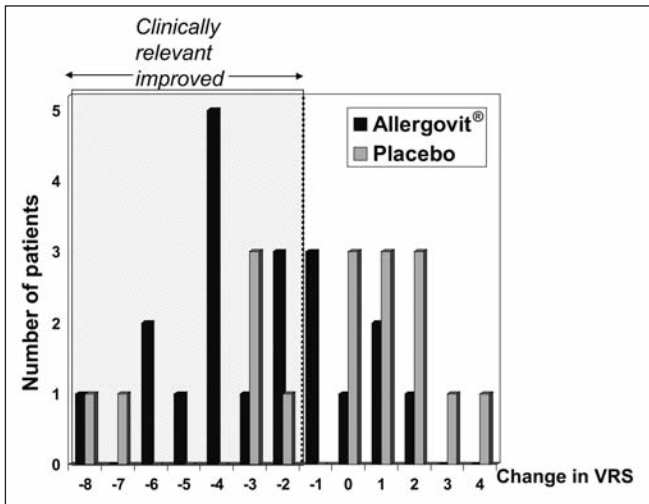


cant difference between both groups ( $p=0.684$ ). After 7 pre-seasonal injections the mean values changed to 4.6 ( $\pm 2.6$ ) for the active group in comparison to 6.4 ( $\pm 3.0$ ) for the placebo group ( $p=0.056$ ). But since it was planned to evaluate non-parametric and a Gaussian distribution was not expected the median change of the VRS was chosen as the primary endpoint. After one pre-seasonal course of SCIT, the VRS showed a difference of -2.5 points as median change in the actively treated group, whereas in the placebo group a median change of 0 point was observed. Taking into account that a reduction of at least two points was defined as significant this clinically relevant parameter showed a statistically significant ( $p=0.024$ ) difference in favour of the active group since 13 of 20 (65%) actively treated patients but only 6/17 (35%) placebo patients improved for at least two points. Figure 3 shows the individual changes for active and placebo treated patients.

The evaluation of the physicians' blinded assessment of the patients' condition as well as of a reduction of anti-symptomatic medication showed a significant improvement for (13/17) actively treated patients against (5/13) placebo patients ( $p<0.05$ ) and a decrease of anti-symptomatic medication for 11/17 patients in the active group against 6/13 placebo patients.

Actively treated patients recorded significant improvement in quality of life with a median increase of 2 and on the other hand, the placebo treated group had a median decrease of 2 for the same ( $p < 0.05$ ).

**Figure 3** - Number of patients with different changes in VRS after one treatment course.



#### Skin prick testing

After one treatment course, threshold concentrations of quantitative prick tests in comparison to 1.0% histamine equivalent were significantly ( $p < 0.05$ ) higher in the active group (median 16,415 SBU/mL before therapy vs. 35,439 SBU/mL after therapy), whereas there was no change after placebo treatment (7,302 SBU/mL vs. 8,849 SBU/mL).

#### Nasal provocation testing

Due to conflicting results in nasal provocation testing before starting SCIT a biometric evaluation of the results was not performed.

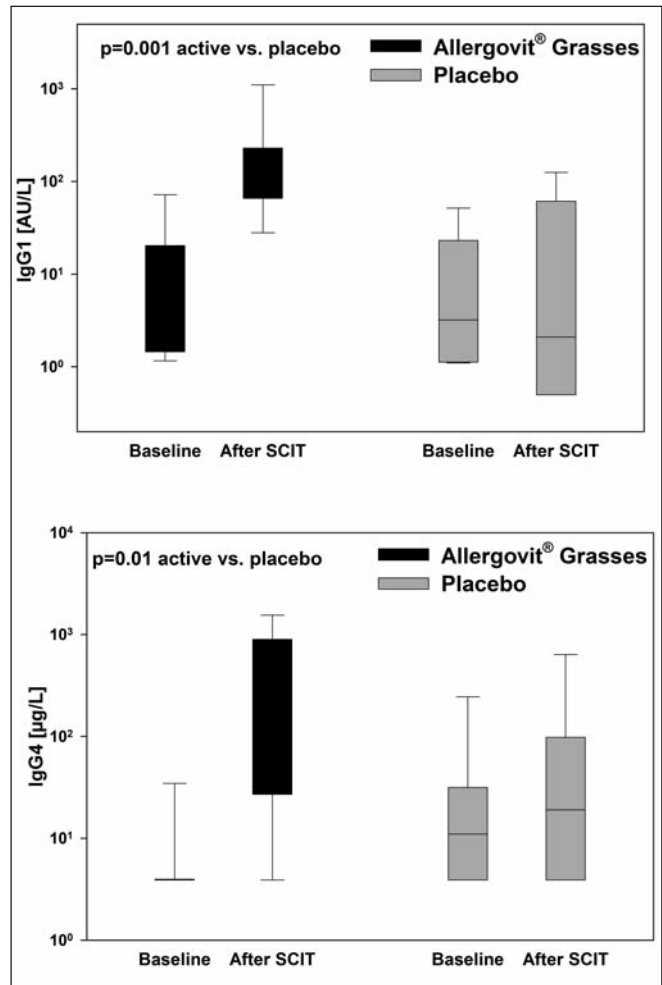
#### Immunological changes

In-vitro measurements of serum samples showed significantly higher values for grass pollen-specific IgG1 ( $p = 0.001$ ) and IgG4 ( $p = 0.010$ ) in the active group after median 7 injections of the grass pollen allergoid (Fig. 4). On the other hand, grass pollen-specific IgE antibody values remained nearly unchanged and comparable in both treatment groups ( $p = 0.869$ ).

#### Tolerability

For the evaluation of tolerance of SCIT, data of the Safety Set were considered. A total of 129 injections were ad-

**Figure 4** - Grass pollen-specific IgG1 (above) and IgG4 (below) median values before and after the pre-seasonal course of Allergovit® Grasses or placebo. The boxes show the median (line), 25th (bottom) and 75th percentile (top), the error bars the 10th (bottom) and 90th percentile (top).



ministered with the active allergoid preparation and 102 with placebo. Local reactions occurred following 88 (68%) injections in actively treated patients and after 40 (39%) injections in the placebo group.

Systemic reactions were graded as follows: mild - eye-itching, sneezing, rhinitis, cough, headache, weariness; moderate - lid edema, sickness, vomiting, diarrhoea, urticaria, wheezing, asthmatic attacks, generalised erythema, generalised pruritus, eczema; strong - reactions which needed emergency treatment (for example antihistamines i.v., corticosteroids i.v., volume substitution, broncholytics, epinephrine i.v.). In the first year of the study, systemic reactions were reported following 14 active injec-



tions (11%) and after 9 placebo injections (9%) and most of the reactions being mild and transient. Four moderate systemic reactions were reported: after 3 active injections urticaria was registered in 3 patients, 1 placebo injection caused edema of the eye lids. No severe systemic and no serious adverse reactions appeared.

## Discussion

Aim of SIT is an immunomodulation that may induce clinical and immunological tolerance as well as long-term efficacy and prevention of progression of the allergic disease when avoidance of the allergen is not possible. To achieve this, allergen doses have to be high enough to achieve the benefit without, however, surpassing the tolerance threshold. Allergoid preparations, in general, are useful to meet this specific need. Next to efficacy and safety, patients' compliance is an important factor which may be improved by the necessity of fewer injections and shorter treatment periods. Results of several clinical studies demonstrate that the aluminium-adsorbed six-grass pollen high-dose hypoallergenic preparation Allergovit® (7, 8, 16, 17) can meet these requirements.

Although the current study on one hand was conducted in one centre, mono-centric studies may have the advantage of a higher conformity in performance of measurements, instructions and data collection. But a mono-centric study may have disadvantages too. The number of patients is relatively small ( $n = 37$ ) and the findings of this study may not necessarily be applicable to other regions. It was planned to include up to 50 subjects to achieve at least 18 evaluable patients per treatment group. In addition, in the case of extreme (low or high) pollen seasons in one centre cannot be balanced by the results of other centres in other regions.

Nevertheless, this study revealed that 65% of those patients receiving active treatment achieved a statistically significant improvement in VRS of well-being already after the first pre-seasonal short-term immunotherapy cycle with 7 injections of depot allergoid. This is noteworthy, because the primary endpoint was evaluated for the ITT population including the 7 patients who should have been excluded from efficacy evaluation due to protocol violations. Some of the patients in the ITT set did not receive at least 5 injections implying less efficacy due to the low cumulative dose.

Further significant and clinically relevant improvement was also shown in terms of beneficial changes of patients' quality of life as assessed by the patients themselves. In addition, assessment by the physician also demonstrated the beneficial

outcome of the pre-seasonal immunotherapy with depot allergoid. Additionally the physician reported that these patients clearly needed less anti-allergic medication during the pollen season, but this data shows that larger sample sizes are necessary to achieve statistical significance. At this point the limitations of a mono-centric trial were evident and a follow-up study which was designed and performed as a multi-centric trial in 154 patients demonstrated statistical significance in this point (7, 8).

The actual "Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases" by the Committee for Medical Products for Human Use (CHMP) in the European Medicine Agency (EMA) which was adopted in November 2008 recommends the use of a combined symptom-medication score as a primary endpoint of a clinical trial (18). In contrast, in this trial a 10-point VAS was chosen as the primary endpoint. Visual analogue scales are quantitative measures largely validated in many diseases and they have been widely used to assess the severity of rhinitis as well as the efficacy of therapeutic interventions (19). In 2003, the joint task force on practice parameters proposed the use of VAS to assess symptom severity of allergic rhinitis (20). Additionally, a recent study on 3052 patients showed that VAS is suitable to quantitatively evaluate severity of allergic rhinitis (19).

An immunologically relevant improvement was also demonstrated already after the first treatment course by a significant reduction in grass pollen skin prick testing and by significant increases of IgG1 and IgG4 antibody titres whilst sIgE titres remained nearly unchanged in both groups. The induction of allergen-specific IgG antibodies, particularly of the IgG4 subclass, as 'blocking antibodies' is the most prominent immunological finding after SIT (21) and the increase in IgG4 was shown to depend on the dose of the SIT preparation (22). Nevertheless, a clear-cut correlation between the serum level of blocking antibodies and the clinical effect of SIT remains to be established (22, 23).

Overall, the benefit-risk ratio is good for the allergoid extract, since no serious adverse reactions occurred. This result is also supported by data from a survey of spontaneous safety reports for Allergovit® (24). Although in this study the number of local reactions seems to be high in both treatment groups, this may be due to recording of even small local reactions (<5 cm in diameter). In general, only local reactions  $\geq 5$  cm are documented (25) and according to the EAACI position paper (4) a dose reduction during SCIT is suggested after local reactions >8 cm. Therefore, the number of local reactions would have been much lower if a 'standard' higher limit had been chosen.

The results of this double-blind, placebo-controlled trial demonstrate that the high-dose hypoallergenic 6-grass pollen preparation is safe and efficacious after one course of a short-term pre-seasonal immunotherapy with seven injections. However, further years of treatment may be required to achieve even more benefit.

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