Abbreviated Clinical Trial Report

E03/05/PP-M

"Evaluation of Immunological Changes after Subcutaneous Immunotherapy with *Phleum pratense* Extract"

Investigational Medicinal Product: Pangramín Depot
Phleum pratense

Clinical trial ID:	E03/05/PP-M
EudraCT No.:	2005-003460-35
Indication:	Allergic rhinoconjunctivitis caused by grass pollen with or without asthma
Development phase:	IV
First subject first visit:	30 November 2005
Last subject last visit:	13 July 2006
Investigators:	[redacted] [redacted] MD, PhD, [redacted] MD, PhD
Trial centres:	[redacted] (Madrid) – Allergy Department [redacted] (Madrid) – Allergy Department
Sponsor:	ALK-Abelló S. A. Medical Department Miguel Fleta 19, 28037 Madrid, Spain
Trial Manager	[redacted] ALK-Abelló, S.A.
Report No. and date:	E03/05/PP-M Abbreviated Clinical Trial Report 2006 Final: 29 December 2006 Updated: 22 March 2007

This trial was conducted in compliance with the principles of *Good Clinical Practice*

SYNOPSIS

Name of Sponsor/Company: ALK-Abelló, S.A.		
Name of Finished Product: Pangramín Depot *Phleum pratense*		
Name of Active Ingredient: *Phleum pratense* pollen allergen extract		

Title of Trial
Evaluation of immunological changes after subcutaneous immunotherapy with *Phleum pratense* extract.

Investigators:
[redacted] (PhD)
[redacted] (PhD)

Trial Centres:
[redacted] Madrid
[redacted] Madrid

Publications:
None.

Trial Period:
First subject first visit: 31 November 2005
Last subject last visit: 13 July 2006

Objectives:
To evaluate the immunological changes to a grass mix pollen extract (*Dactylis, Festuca, Lolium, Phleum, Poa*), after subcutaneous immunotherapy with *Phleum pratense*. As markers of immunological changes, the immediate and late-phase skin reactivity and the levels of specific immunoglobulins against grass mix and *Phleum pratense* allergens extracts were studied.

Methodology:
An open, randomized, controlled, parallel group, multi-centre clinical trial. The study was carried out in the Allergy Department of two Spanish Hospitals.
Patients were randomized to receive a course of immunotherapy with *Phleum pratense* extract for 3-4 months, or to a control group, without immunotherapy.
Immunological evaluations were performed before administering the first dose of *Phleum* SIT and at the end of the treatment.
46 patients were randomized 3:1 to receive either treatment with *Phleum pratense* or being control.
Along the trial, patients visited the centre in four occasions (selection, randomization, end of treatment and final visit). In addition, patients who received Phleum SIT had the necessary visits to administrate the treatment in the active group: four weekly visits in the up-dosing period followed by one fortnightly visit and 2-3 monthly administrations.

Number of Subjects Planned and Analysed:
50 planned.
46 included, 46 randomised.
33 treated in immunotherapy group, 13 in control group.
38 completed, 25 in the SIT group, 13 in the control group.
8 withdrawn: 3 due to withdrawal of consent, 3 lost to follow-up, 1 adverse event, 1 other reasons
46 subjects analysed for safety, 38 for efficacy

Diagnosis and Main Inclusion Criteria:
Rhinoconjunctivitis due to sensitisation to grass pollens with or without asthma.
Patients with age between 12 and 55 years old and with positive skin prick tests to grass pollen mix extract were included.
Patients with FEV_1 < 80% of predicted value, pregnant women, asthma and/or severe atopic dermatitis, previous immunotherapy with grass pollen extracts in the last 5 years or contraindications

of immunotherapy and/or any clinical disease which may affect the results of the study or compromise patient's safety, were excluded.

Investigational Medicinal Product, Dose and Mode of Administration, Batch Number:
Phleum pratense allergen extract, biologically standardized and with its major allergen Phl p 5 quantified in mass units and adsorbed to aluminium hydroxide (Pangramín Depot *Phleum pratense*) for subcutaneous immunotherapy.
Active treatment was administered following a clustered build-up schedule of four weekly visits, and continued with one fortnightly dose and 2-3 monthly maintenance administrations. The monthly maintenance dose was 2 µg Phl p 5. Batch numbers were EC-Z221 for vial 2 (0.25 µg/ml Phl p 5) and EC-Z220 for vial 3 (2.5 µg/ml Phl p 5).

Reference Therapy Dose and Mode of Administration, Batch Number:
Subjects in the control group were treated with symptomatic drugs, without *Phleum* SIT.

Duration of Treatment:
Average duration of the immunotherapy treatment for subjects who completed the treatment was 3.6 months (min 2.1, max 5.1 months).

Criteria for evaluation of efficacy:
The main efficacy variable was the evaluation of changes in the immediate skin reactivity (wheal area) 15 minutes after skin prick test with 4 five-fold dilutions in duplicate of the grass mix pollen extract, analyzed by Parallel Line Assay (PLA).
The secondary efficacy variables were the immediate cutaneous response to *Phleum pratense*, the late-phase skin reaction 6 hours after intradermal skin tests with grass mix and *Phleum pratense* pollen extracts and the evaluation of the levels of IgE and IgG_4 to grass mix and *Phleum pratense* pollen extracts, measured before and after treatment.

Criteria for evaluation of safety:
Adverse events (AEs) were the safety parameter evaluated.

Statistical Methods:
Sample size was estimated from previous studies of immunotherapy in which skin tests responses were evaluated by parallel line bioassay (PLA Martín S, Cuesta P, Rico P, Cortés C. A computer program based on parallel line assay for analysis of skin tests. Allergy 1997;52:97-100) showing that a reduction between 2 and 10 times can be expected. With an estimated effect size of 1.0, 44 patients distributed in two groups of 11 and 33, will have a 80% statistical power to detect differences with a two-sided test at a 5% significance level. Accounting for a 10% sample loss, the number of patients to be included is 50. Randomization of patients into the active and control groups followed a 3 to 1 ratio.

The following analysis sets were used:
• *Full-Analysis Set* (FAS) – comprising all subjects randomised following the Intention to Treat (ITT) ICH principle. The FAS was the primary set for safety analysis.
• *Per-Protocol Set* (PP) – comprising subjects who completed the protocol without major protocol deviations.
The evaluation of immediate cutaneous response was performed by means of the parallel line assay. For the delayed cutaneous response and specific immunoglobulins, the Student t-test was used.
AEs were classified and summarised according to MedDRA.

Demography of Trial Population:
Both groups were similar in their demographics and clinical parameters. Some non-significant differences were observed in the severity of rhinitis.

		Active		Control		Total		
		N	%	N	%	N	%	p value[1]
Number of Subjects		33	71.7	13	28.3	46	100.0	
Sex	Male	13	39.4	6	46.2	19	41.3	0.746
	Female	20	60.6	7	53.8	27	58.7	
Asthma	Absent	13	39.4	7	53.8	20	43.5	0.547
	Mild-Int	16	48.5	5	38.5	21	45.7	
	Mild-Pers	3	9.1	0	0.0	3	6.5	
	Moderate	1	3.0	1	7.7	2	4.3	
	Severe	0	0.0	0	0.0	0	0.0	
Rhinitis	Absent	0	0.0	0	0.0	0	0.0	0.096
	Mild-Int	11	33.3	2	15.4	13	28.3	
	Mild-Pers	12	36.4	2	15.4	14	30.4	
	ModSev-Int	5	15.2	6	46.2	11	23.9	
	ModSev-Pers	5	15.2	3	23.1	8	17.4	
Conjunctivitis	Absent	3	9.1	0	0.0	3	6.5	0.204
	Mild	18	54.5	4	30.8	22	47.8	
	Moderate	11	33.3	8	61.5	19	41.3	
	Severe	1	3.0	1	7.7	2	4.3	
Age (years, mean ± sd)		31.4 ± 7.3		29.5 ± 6.1		30.9 ± 7.0		0.417

[1]Chi-square, except Age (t Student)

Efficacy Results:

Immediate cutaneous response:
The active group showed a significant decrease in the reactivity towards the grass mix extract (CTI = 2.93, 95% CI [2.03 – 4.23]) and towards the *Phleum pratense* extract (CTI = 3.45, 95% CI [2.22 – 5.37]). The control group did not show significant changes neither with the grass mix extract, nor with the *Phleum pratense* extract. The comparison of the skin response before SIT was similar for both groups with both allergen extracts but after SIT, due to the decrease in cutaneous sensitivity, the active group had a significant lower response to the grass mix and *Phleum pratense* extracts than the control group. There were not differences in the comparison of the response to the grass mix extract versus the *Phleum pratense* extract before or after SIT.

Delayed cutaneous response:
The late phase response showed a significant decrease in the active group after the intradermal test with grass mix and *Phleum pratense* ($p<0.001$) not apparent in the control group. Before SIT, active and control groups had similar delayed response to both allergen extracts but, after SIT, the size of the delayed response to grass mix and *Phleum pratense* allergen extracts was significantly lower in the active group ($p=0.022$ for grass mix and $p=0.028$ for *Phleum*). There were not differences in the comparison of the response to the grass mix extract versus the *Phleum pratense* extract before or after SIT.

Specific immunoglobulins:
Specific IgE towards grass mix and *Phleum pratense* increased in a significant way from before SIT to after SIT, not only in the active group ($p<0.001$ for both extracts) but also, although to a lower extent, in the control group ($p=0.010$ for grass mix, $p=0.012$ for *Phleum*). In spite of these changes, the levels of IgE were not statistically different between groups, neither before nor after SIT, and the comparison of these changes between groups were also non significant.

Specific IgG4 to grass mix and *Phleum pratense* showed an important increase ($p<0.001$) in the active group and, to a lower extent, also in the control group ($p=0.047$ for grass mix and $p=0.036$ for

Phleum). The comparison of IgG4 levels between the active and control groups was non-significant before SIT but very significant after SIT ($p<0.001$). In addition, the change in specific IgG4 was statistically different in the active group compared to the control group for grass mix and *Phleum pratense* extracts. The IgG4 response after SIT seems to be biased preferentially towards the *Phleum pratense* extract rather than to the grass mix extract.

Safety Results:

A total of 317 SIT doses were administered to 30 patients with an average of 11.4 doses administered (min 10, max 14) to the 25 patients which completed the study.

37 adverse events were reported in 17 patients, all in the active group. 22 (59%) of them were not related to the treatment and 29 (78%) were mild in severity, 7 (19%) moderate and 1 (3%) severe. All patients were recovered and there was not any severe adverse event.

N = 37	Mild		Moderate		Severe		Total		Total
	NR	Related	NR	Related	NR	Related	NR	Related	R + NR
Eye disorders		2 (5.4)	1 (2.7)	1 (2.7)			1 (2.7)	3 (2.7)	4 (10.8)
Immune system disorders						1 (2.7)		1 (2.7)	1 (2.7)
Infections and infestations	2 (5.4)		1 (2.7)				3 (2.7)		3 (2.7)
Metabolism and nutrition disorders	1 (2.7)						1 (2.7)		1 (2.%)
Musculoskeletal and connective tissue disorders	1 (2.7)						1 (2.7)		1 (2.7)
Nervous system disorders	6 (16.2)						6 (16.2)		6 (16.2)
Respiratory, thoracic and mediastinal disorders	4 (10.8)	1 (2.7)	2 (5.4)	1 (2.7)			6 (16.2)	2 (5.4)	8 (21.6)
Skin and subcutaneous tissue disorders	2 (5.4)						2 (5.4)		2 (5.4)
General disorders and administration site condition	2 (5.4)	8 (21.6)		1 (2.7)			2 (5.4)	9 (24.3)	11 (29.7)
Grand Total	18 (48.6)	11 (29.7)	4 (10.8)	3 (2.7)		1 (2.7)	22 (59.5)	15 (40.5)	37 (100)

Number of events and (%); NR, not related

The 15 adverse reactions (related to the treatment) appeared as a consequence of 10 doses administered in 9 patients (30%). 4 (27%) of them were treated and the severity was mild in 10 (73%), moderate in 3 (20%) and severe in 1 (7%) of them. In 6 of the doses with AR the administration schedule did not changed, in 2 the dose was reduced, in 1 there was a temporally interruption and in another one the treatment was discontinued. All the AR appeared during the updosing phase.

7 AR consisted in local reactions, 4 in upper respiratory symptoms (rhinoconjunctivitis), 1 in lower respiratory symptoms (cough), 2 in unspecific symptoms (tiredness) and 1 in anaphilaxia with asthma and itching in the palate and ears. The frequency of AR was 3.15% of the doses. All of the AR were resolved.

Conclusions:

The study shows that a short course of subcutaneous immunotherapy with a standardised *Phleum pratense* allergen extract is able to promote immunological changes towards a mix of grass allergens related to *Phleum pratense*. These changes have been observed in all of the evaluations performed and with only 3-4 months of pre-seasonal SIT. Although the study has not been designed for the comparison of the responses towards *Phleum pratense* and grass mix, the results suggest that both responses are similar, at least with regards to the cutaneous response, early and delayed.

Overall, the rate of adverse reactions has been low, accounting those of a systemic outcome less than 2% of the doses. All the adverse reactions have appeared during the updosing phase and this is in accordance with the published data on tolerability of subcutaneous SIT and with the common practice. There has not been any serious adverse event and all the adverse reactions were resolved spontaneously and only 27% received treatment. One patient was withdrawn because an adverse reaction.

Date of the Report:

29 December 2006

This trial was conducted in compliance with the principles of *Good Clinical Practice*.