1. SYNOPSIS

Name of Sponsor:
Laboratorios LETI, S.L.
Name of finished product:
Depigoid
Name of active ingredient:
Allergen extract of Parietaria judaica

Title of study:	Clinical, Multi-centre, Randomised, Double-blind Placebo-controlled Subcutaneous Immunotherapy Trial with Depigmented, Polymerized Extract of Parietaria judaica on Patients with Hypersensitivity to this Pollen.
Investigators:	There were a total of 9 investigators with Dr. A. Ferrer Torres from Vega Baja Hospital acting as principal coordinator
Study centre(s):	There were 10 active centres where patients were recruited in Spain
Publication (reference):	None
Studied period (years):2 years **(date of first enrolment):** 09/04/2008 **(date of last completed):** 28/02/2013	**Phase of development:** III
Objectives:	**Primary:** The primary objective was to assess the clinical efficacy of the depigmented, glutaraldehyde-polymerized allergen extract of *Parietaria judaica* pollen in the treatment of patients affected by allergic rhinitis/rhinoconjunctivitis induced by hypersensitivity to this pollen, evaluating the Combined Symptoms and Rescue Medication Score. **Secondary:** To evaluate the effect of the treatment on the following parameters: • Quality of life • Prick-test dose response • Inflammatory markers ECP in blood Cytokines in blood Exhaled nitric oxide • Nasal provocation test • Adverse events • Use of health resource
Methodology:	Multi-centre, prospective, double-blind, randomised, placebo-controlled clinical trial, with two treatment groups. **Experimental Group –** This group received depigmented, glutarladehyde-polymerized allergen extract of *Parietaria judaica* pollen subcutaneously. Depigoid Parietaria judaica 1000 DPP/mL as a maintenance dose. **Control Group -** This group received subcutaneously identical solution to the investigational medicinal product but without active ingredients. Both patient groups were subjected to environmental control measures and optimum pharmacological treatment to control their rhinitis or rhinoconjunctivitis and bronchial asthma that may have appeared during the trial, according to the recommendations of the ARIA guidelines and GINA2006.
No. of patients: **Planned:**	148

Analysed:

	Experimental drug	Placebo	Total
Screened			152
Randomized	70 (100.0%)	76 (100.0%)	146 (100.0%)
Safety	69 (98.6%)	75 (98.7%)	144 (98.6%)
Intent-to-treat	62 (88.6%)	61 (80.3%)	123 (84.2%)
Per Protocol	60 (85.7%)	60 (78.9%)	120 (82.2%)

Diagnosis and main criteria for inclusion:

Patients, aged between 18 and 55, affected by rhinitis or rhinoconjunctivitis induced by hypersensitivity to Parietaria pollen.

- Informed consent signed by the patient.
- Aged between 18 and 55.
- Clinical history of rhinitis or symptomatic allergic rhinoconjunctivitis not previously diagnosed, due to allergy to Parietaria pollen.
- Sensitisation to *Parietaria* pollen, diagnosed by:
  a) Positive specific nasal provocation test;
  b) Positive skin tests for *P. judaica* (the papule produced by the prick test must be equal to or greater than the papule produced by the positive control [histamine 10 mg/ml]); and
  c) Presence of specific IgE for *P. judaica* >0.7 kU/L.

  The patients included had to be monosensitised or, in the case of polysensitised patients, sensitisation to *P. judaica* pollen was considered the only one that was relevant to their disease from a clinical point of view during their period of inclusion in the trial.

Test product: Depigoid® of *Parietaria judaica* vial no. 2 (1,000 DPP/ml) (depigmented allergen extract, polymerized with glutaraldehyde and adsorbed onto aluminium hydroxide)

dose: Starting dose: 0.2 ml + 0.3 ml the first day
Maintenance dose: 0.5 ml
The starting dose of 0.2 ml of active substance or placebo (visit 1) was administered subcutaneously into one arm, followed by 0.3 ml in the other arm 30 minutes later. The maintenance stage, from visit 2 to visit 24, consisted of the monthly administration of 0.5 ml of active substance

mode of admin.: Subcutaneous

Duration of treatment: 24 months per patient.

Reference therapy: Placebo

dose: Identical solution to the experimental product but without active ingredients.

mode of admin.: Subcutaneous

Criteria for evaluation:

Efficacy:

The primary efficacy outcome was the combination of scoring of symptoms and rescue medication for rhinitis and rhinoconjunctivitis evaluated in the second pollen season. The combined scoring of symptoms and drugs represented the sum of the area under the curve (AUC) weighted on the basis of the time of the daily combined scoring of the burden of the symptoms and use of rescue medication among the treatment group and the placebo group evaluated in the second pollen season and pollination peak. In order to comply with the "Guideline on the requirements to the clinical development of products for specific immunotherapy for the treatment of allergic diseases", 6 symptoms were evaluated for the scoring of rhinitis and rhinoconjunctivitis: itchy nose, sneezing, rhinorrhoea, nasal obstruction, eye itching/redness/foreign body sensation and tearing. The symptoms eye itching, redness and foreign body sensation were rescored as one single symptom (eye itching/redness/foreign body sensation) taking the highest daily score of the three symptoms for calculation of the combined symptom and drug score. The maximum score for the drugs for rhinitis and rhinoconjunctivitis was 18 points and was weighted against the symptoms.

The following parameters were evaluated:

1. The combined symptom and rescue medication score for rhinitis and rhinoconjunctivitis evaluated after one season. The combined symptom and rescue medication score represent the sum of the time weighted AUC of daily symptom severity scores and the time weighted AUC of daily rescue medication score. The combined symptom and rescue medication score within the timeframe of the predefined pollen season and peak pollen will be taken into account (evaluated after one season of treatment). NOTE: Rhinitis/rhinoconjunctivitis symptoms are nasal itching, sneezing, rhinorrhea (runny nose), nasal obstruction (Nasal congestion), ocular tearing, Ocular itching/redness/grittiness.
2. The combined symptom and rescue medication score for rhinitis, rhinoconjunctivitis and bronchial asthma evaluated after one and two seasons of treatment in asthmatic subjects during the pollen season and peak pollen. The combined symptom and rescue medication score represent the sum of the time weighted AUC of daily symptom severity scores and the time weighted AUC of daily rescue medication score. The combined symptom and rescue medication score within the timeframe of the predefined pollen season and peak pollen will be taken into account (evaluated after one and two seasons of treatment). NOTE: Rhinitis/rhinoconjunctivitis and bronchial asthma symptoms are nasal itching, sneezing, rhinorrhea (runny nose), nasal obstruction (Nasal congestion), ocular tearing, Ocular itching/redness/grittiness, cough, wheezing and dyspnoea.
3. Symptom Score for rhinitis/rhinoconjunctivitis during the pollen seasons.
4. Symptom Score for rhinitis/rhinoconjunctivitis and bronchial asthma symptoms during the pollen seasons in asthmatic subjects.
5. Rescue medication score for rhinitis/rhinoconjunctivitis during the pollen.
6. Rescue medication score for rhinitis/rhinoconjunctivitis and bronchial asthma during the pollen seasons in asthmatic subjects.
7. Combined symptom and all rescue medication score for bronchial asthma (cough, wheezing and dyspnoea) during the pollen seasons in asthmatic subjects.
8. Individual symptom scores: Nasal itching, Sneezing, Rhinorrhea (runny nose), Nasal obstruction (Nasal congestion), Ocular itching/redness/grittiness, Ocular tearing, Cough, Wheezing and Dyspnea during the pollen season.
9. Symptom score for nasal symptoms (Nasal itching, Sneezing, Rhinorrhea (runny nose), Nasal obstruction (Nasal congestion)) during the pollen season.
10. Symptom score for ocular symptoms (Ocular itching/redness/grittiness, Ocular tearing) during the pollen seasons.

11. Symptom score for bronchial symptoms (asthma Cough, Wheezing and Dyspnea) during the pollen seasons.
12. Rescue medication score classified by pharmacological family and same score during the pollen seasons of each investigational site.
13. Well days for rhinitis/rhinoconjunctivitis: Number and proportion of subjects with a symptom score ≤2 and no rescue medication for rhinitis/rhinoconjunctivitis during the pollen seasons.
14. Well days for rhinitis/rhinoconjunctivitis and bronchial asthma; Number and proportion of subjects with a symptom score ≤2 and no rescue medication for rhinitis/rhinoconjunctivitis and bronchial asthma, during the pollen seasonsin asthmatic subjects.
15. Hell days for rhinitis/rhinoconjunctivitis: Number and proportion of subjects with a symptom score ≥10 and taking rescue medication in the pollen season during the pollen seasons.
16. Hell days for rhinitis/rhinoconjunctivitis and bronchial asthma: Number and proportion of subjects with a symptom score ≥10 and taking rescue medication, in the pollen season during the pollen seasons in asthmatic subjects.
17. Medication free days for rhinitis/rhinoconjunctivitis; Number and proportion of subjects with no rescue medication during the pollen seasons.
18. Medication free days for rhinitis/rhinoconjunctivitis and bronchial asthma; Number and proportion of subjects with no rescue medication during the pollen seasons in asthmatic subjects.
19. Rhinoconjunctivitis Quality Of Life Questionnaire (RQLQ).
20. Use of health resources
21. Exhaled nitric oxide
22. Respiratory function spirometry
23. Specific nasal provocation test
24. Prick test dose-response
25. Serology:
    - Total IgE
    - Specific IgE
    - Specific IgG, IgG1and IgG4
    - Ratio Specific IgE/Specific IgG4
    - IFN-γ
    - IL-4
    - IL-10
    - ECP
26. Number and proportion of subjects who developed asthma in each treatment group.
27. Pollen count per day per season and per region.

Safety:

1. Adverse events

Statistical methods:

The statistical tests performed were the following:

1.- Descriptive statistics (arithmetic mean, standard deviation, variance, standard error, etc.) of each of the results, and the percentages in the qualitative variables.

The confidence interval for safety of the primary and secondary variables was 95%.

2.- Inter-group comparisons:

- The χ^2--square test, or Fisher exact test if necessary, was applied in the qualitative variables. In addition, a logistical regression model was applied on an exploratory basis, with variable efficacy as the dependent element and the treatment group as the independent element. This model included other co-

variables to detect potential confusion factors, including the use of rescue medication (section 7.10 of the protocol).

- For the quantitative variables, including the primary variable, the appropriate parametric techniques was used first (Student's t). The non-parametric techniques (Mann Whitney test) were used, depending on the result of the positive Gaussian curve adjustment test (Shapiro Wilk's test). In addition, an ANCOVA was performed to control other factors, which included both the final score value and the baseline value of the symptoms score and the permitted use of rescue medication, as indicated in section 7.10 of the protocol.

SUMMARY - CONCLUSIONS:

Out of the 152 screened patients, 146 (96.1%) were randomized and treated, out of these randomized patients, 123 patients (84.2%) were included in the ITT population (62 in the Depigoid arm and 61 in the placebo arm). The PP population consisted of 120 patients (82.2%) distributed in 60 patients in the Depigoid arm and 60 in the placebo arm. The safety population included the 144 randomized patients. The distribution of patients in the ITT population at baseline was almost half males and females (40.3% and 52.5% respectively). Almost all of them were of Caucasian origin (95.9%) and mean ± SD age was of 38.0 ± 10.2 years. IgE specific *Parietaria judaica* levels at baseline were slightly higher in the placebo group (30.7 ± 36.7 KU/L and 21.1 ± 22.1 KU/L for placebo and Depigoid respectively).

Efficacy results:

Median injections before first pollen season was 3 (IQR 2, 4) in both groups. According to previously established median injections in other studies, a minimum number of 5 injections are needed to evaluate efficacy. Only 4 patients in Depigoid Parietaria group and 5 in placebo group reached that number. No conclusions can be obtained, in terms of efficacy in the first pollen season. 90% of the subjects received 16 doses before second pollen season (no differences between groups).

Primary outcome:

The time weighted AUC mean for combined symptom and rescue medication score for rhinitis/rhinoconjunctivitis after two seasons was higher in the Depigoid group (3.65) compared to the placebo group (2.78) during the pollen season without reaching statistical significance (p=0.0647). Differences reach significance during peak pollen season in favour of placebo group (0.0380). Same trend –placebo group scores lower- is observed when looking at individual scores.

Other outcomes:

- Regarding changes of immunoglobulins parameters from baseline, for specific IgE in KU/L, after the treatment no differences were found for the Depigoid Parietaria group (from 21.06 to 22.59) compared with Placebo group (from 30.67 to 28.60). There was a significant increase (from 0.58 to 1.04) after the treatment in the active group, not observed in placebo for the specific IgG4 in mg/L (from 0.85 to 0.70). Also, differences in IgE/IgG4 ratio were obtained between groups in the same period, ratio decreases in Depigoid Parietaria group (from 165.31 to 73.95) but not in placebo group (from 195.41 to 227.91). Finally, no differences were found in other laboratory parameters: ECP, IFN-γ, IL-4 and IL-10.
- The percentage of patients who increased in the value concentration which produced a papule size greater than 7 mm2 from baseline visit to end of treatment, in the prick test dose-response, was higher in Depigoid group (20.0%) than in placebo group (0.0%).
- An increase in active group of 64.9% in the value of the concentration which produced a positive specific nasal provocation test from baseline visit to end of treatment, was higher than in placebo group (41.4%).
- No differences were found in the rest of the secondary outcomes.

Safety results:

- Number of patients with ADRs during the study period were similar in Depigoid group (4 out of 69) compared to placebo group (5 out of 75).
- Local and systemic reactions were of mild to moderate intensity, and reactions did not lead to dose reduction, interruption or discontinuation in any case.
- Local reactions were late reactions and injection site reactions in both treatment groups.
- Systemic reactions were very low, one patient in the Depigoid group and 2 patients in the placebo group reported a systemic reaction.
- Regarding adverse events, the most frequently reported were infections and infestations which were suffered by 39.1% and 32.0% in the Depigoid and placebo groups respectively, followed by musculoskeletal and connective tissue disorders 10.1% and 6.7%.
- There were 3 patients who reported 4 SAEs in the Depigoid group and 1 patient who reported 1 SAEs in the placebo group. None of the SAEs was related to the study medication in any of the treatment groups.
- One patient withdrew the study due to a TEAE (treatment emergent adverse event) in the Depigoid group (Decreased appetite and depressed mood) and one patient in the placebo group (Vaginal hemorrhage).There were statistical differences between treatment arms in changes from baseline to the end of the study of systolic and diastolic blood pressure and heart rate.

Discussion and Overall Conclusions:

The study was designed to assess the clinical efficacy of the depigmented glutaraldehyde polymerized allergen extract Depigoid *Parietaria judaica* 100%, in the treatment of patients affected by rhinitis/rhinoconjunctivitis induced by hypersensitivity to P. judaica pollen, evaluating the combined symptoms and rescue medication score.

Patients who were included in the study had to be aged between 18 and 55 years, suffering from rhinitis or rhinoconjunctivitis induced by hypersensitivity to P. judaica pollen. Patients included should be monosensitised or, in the case of polysensitised patients, sensitisation to P. judaica pollen should be considered the only one that was relevant to their disease from a clinical point of view during their period of inclusion in the trial.

With these premises, a total of 152 patients were screened and 146 of them were randomized to receive one of the two treatment (70 patients in the Depigoid group). The ITT population was made up of 123 patients and 3 more patients were excluded from the PP population.

The distribution of patients at baseline was almost half males and females (46.3% and 53.7%, respectively). Almost all of them were of Caucasian origin with a median age of 39.21 years (IQR 30.20 – 44.44). Although IgE specific for P. judaica levels at baseline were slightly higher in the placebo group, differences were neither clinical nor statistically significant, so the treatment groups were well distributed and with unbiased baseline data.

The present study failed in showing expected clinical benefit of the allergen extract Depigoid Parietaria since the combined symptom and rescue medication score for rhinitis/rhinoconjunctivitis, which was the primary objective of the study, was higher in the Depigoid group compared to the placebo group after two years of treatment. The same trend was observed when looking at individual symptom and rescue medication scores (even individual symptoms). Similar results were found for the PP population.

Several actions were taken to confirm such results: quality of the data and statistical analysis were confirmed, quality of diaries and database was assured and an independent statistician re-analyzed the data confirming same result and active/placebo balance in study sites was correct.

Several factors such as patient selection, co-sensitization to other allergens, extension of recruitment period or variability in pollen count and scores among centers, may have influenced these results.

Although the indication for allergen immunotherapy is moderate to severe rhinitis or rhinoconjunctivitis, this assessment was not considered as inclusion criteria. This could have lead to inclusion of mild patients. When looking at the primary variable both active and placebo groups had low scores. These data together with the number of well and hell days, suggest that mild patients were included.

Regarding co-sensitization, patients were selected according to a suggestive clinical history and positive skin prick test to P. judaica and P. judaica specific IgE > 0.7 KU/l. In the event of co-sensitization, a patient was allowed to participate if it was considered not relevant. Relevancy was established based on clinical symptoms reported by the patient without any further confirmation. Due to the extension of recruitment period over one year, it was necessary to include different years in the analysis increasing the variability in pollen counts.

Field studies are highly dependent on environmental conditions and pollen counts that condition symptoms severity in patients. In this particular clinical trial, recruitment period was extended longer than expected and different patients were exposed to different pollen seasons, not homogenous among them. Allergen exposure is usually assessed by pollen counts, but may misrepresent exposure if performed remotely from multiple study centers, which was the case of this study. Behavior of centers was not homogenous, there were differences in pollen counts and symptom scores; in fact in two of the study sites, positive differences in favour of the active treatment were recorded.Evaluation of symptoms and medication score was based on paper patient diaries. Monitoring, compliance and accuracy of those diaries can be improved using electronic diaries in future trials, which was not the case for this study. Additionally, according to the EAACI position paper recently published (Pfaar et al. Allergy; 2014; 69: 854-867), the use of rescue medication should be standardized and using a stepwise approach. For this reason, in future trials rescue medicaction should be provided to study participants. Study medication was not provided in this study.

Differences were found in specific IgG4, ratio IgE/IgG4 in the Depigoid group after treatment, but this subtle increase cannot be considered clinically relevant. Thus, the expected immunological changes for allergen immunotherapy, namely an increase in allergen-specific IgG4, could not be confirmed in this study. In addition there were no changes for other laboratory parameters: ECP, IFN-γ, IL-4 and IL-10.

Regarding other secondary endpoints such as SPT dose-response and specific NPT statistically significant differences were found, in favour of the actively treated patients. This supports the immunological effect of the treatment, and support its efficacy. Although statistically significant differences were found in NPT, the clinical relevance is limited.This may be due the technique itself – because of lack of reproducibility-, and to the fact that collaboration of the patient as well as experienced staff performing the test are needed.

No differences were found between groups in the RQLQ questionnaire after treatment. It is important to consider that median values in both groups at baseline and within pollen seasons (first and second) was around 1.5 in both groups, which means that subjects in this study suffered from very mild allergy symptoms. This finding correlates with symptoms scores as previously mentioned.

Regarding safety, local and systemic reactions were of mild to moderate intensity. Local reactions were late and injection site reactions in both treatment groups. Systemic reactions were very low. No more than one patient in the Depigoid group reported a systemic reaction, whereas in the placebo group 2 patients reported conjunctivitis.

The most frequently adverse events reported were infection and infestations in both treatment groups. There were 3 patients who reported 4 SAEs in the Depigoid group and 1 patient who reported 1 SAE in

the placebo group. None of the SAEs were related to the study medication or study procedures in any of the treatment groups.

A previous efficacy study was performed with this medicinal product and results allowed to conclude that Depigoid Parietaria was efficacious and safe in the treatment of patients suffering from allergic rhinitis-rhinoconjunctivitis.

In conclusion, the expected (and confirmed in a previous study) clinical effect could not be concluded in the present study. This most likely resulted from poor patient selection (inclusion of patients with few symptoms), too long extension of recruitment period, variability in pollen count and scores among centers, and also possibly inadequate dose of immunotherapy. We consider that additional studies to generate efficacy data of this product are needed, including higher doses.

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