## 1. TITLE PAGE

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| STUDY TITTLE | Biological standardization of *Cupressus arizonica* allergen extract to determine the biological activity in HEP units. | |
| PROTOCOL NUMBER | 608-PR-PRI-199 | |
| EUDRACT NUMBER | 2013-001309-98 | |
| INVESTIGATIONAL MEDICINAL PRODUCT | Experimental product:<br>*Cupressus arizonica* allergen extract<br>Control product:<br>Histamine dihydrochloride (10 mg/ml)<br>Glycerinated phenol saline solution | |
| INDICATION STUDIED | Diagnosis of *Cupressus arizonica* allergy | |
| DESIGN | Non-randomised, open-label and multi-center clinical trial. | |
| SPONSOR | Laboratorios LETI, S.L. Unipersonal<br>C\ Sol, 5. 28760 Tres Cantos. Madrid. Spain<br>Phone: +34 91 7711790// Fax: +34 91 8040919//www.leti.com | |
| PHASE OF STUDY | II | |
| STUDY INITIATION DATE | 22nd of July 2015 | |
| STUDY COMPLETITION DATE | 24th of December 2015 | |
| COORDINATING INVESTIGATOR | Not applicable | |
| PRINCIPAL INVESTIGATOR | Dra. Matilde Rodríguez Mosquera<br>Servicio de Alergología<br>Hospital Puerta de Hierro-Majadahonda<br>C/ Manuel de Falla, 1,<br>28222 Majadahonda, Madrid (Spain) | Dra. Eloina González Mancebo<br>Unidad de Alergología<br>Hospital Universitario de Fuenlabrada<br>C/ Camino del Molino, 2<br>28942 Fuenlabrada Madrid (Spain) |
| MEDICAL DIRECTOR LETI GROUP | Jaime Sánchez, MD, PhD | |
| GCP STAMENT | This study was performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements | |
| ARCHIVING | The study documents will be archived according to ICH GCP regulations | |
| DATE OF REPORT | 06/AUG/18 | |

For any questions of the study report, please contact to Medical Department, Laboratorios LETI S.L.U (+34 91 7711790).

SYNOPSIS

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| **Name of Sponsor / Company:** Laboratorios LETI, S.L.Unipersonal | |
| **Name of Finished Product**: | Prick Test *Cupressus arizonica* LETI at 15, 10, 5 and 1 mg/ml<br>Positive control (histamine dihydrochloride {10 mg/ml})<br>Negative control (glycerinated phenol saline solution) |
| **Name of Investigational product substance(s):** | Experimental product:<br>*Cupressus arizonica* allergen extract<br>Control product:<br>Histamine dihydrochloride (10 mg/ml)<br>Glycerinated phenol saline solution |
| **Name of Non Investigational product substance:** | Prick Test de *Cupressus arizonica* 30 HEP/ml |

**Title of the study:** Biological standardization of *Cupressus arizonica* allergen extract to determine the biological activity in HEP units.

**Investigator(s):**


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**Study centres:** 2 sites in Spain

**Publication** (reference): None at the time of this clinical study report (CSR)

**Study period**:
First site initiated: 21st of September 2015
100% sites initiated: 30th of November 2015
First site closed: 30th of November 2015
100% sites closed: 24th of December 2015
First patient first visit: 21th of September 2015
Last patient last visit: 30th of November 2015

**Study phase**: II

**Objectives:** The objective of this study is to determine the biological activity of a *Cupressus arizonica* allergen extract in histamine equivalent prick (HEP) units, in order to be used as in-house reference preparation (IHRP).

**Methodology**: Titrated Skin Prick test biological study. Four concentrations of *Cupressus arizonica* allergen extract, together with a positive and negative control, were tested in every patient in duplicate on

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the volar surface of the forearm. The tests were performed at the study sites and all patients remained in the medical rooms under observation at least 30 minutes after the application of the Titrated Skin Prick test.

**Number of patients (planned and analysed):** A total number of 33 patients were enrolled and received the study medication, but 13 of them were excluded from the PP population since they did not meet the Nordic Guidelines (1) statistical criteria for analysis. The wheal data of 20 patients were analysed (PP population).

**Diagnosis and main criteria for inclusion and exclusion**:

- Main patient inclusion criteria:
  1. Patient has provided written informed consent, appropriately signed and dated by the patient (or legal representative, if applicable).
  2. Patient can be male or female of any race and ethnic group.
  3. Age $\geq$ 18 years and $\leq$ 60 years at the study inclusion day.
  4. Positive skin prick test with a standardised commercially available preparation of *Cupressus arizonic*a allergen extract. The skin prick test will be considered positive if the test results in a wheal major diameter of at least 3 mm or wheal area at least 7 $mm^2$. Positive skin prick test results should be performed during the selection day (at least 30 minutes before the Prick tests and in an area above to that to be used for the Prick test response).
  5. A positive test for specific IgE to *Cupressus arizonic*a (CAP-RAST $\geq$ 2). IgE results are valid if performed within one year prior to the inclusion of the patient in the study.
  6. Positive clinical history of respiratory allergy (rhinitis and / or rhinoconjunctivitis and / or asthma) against *Cupressus arizonica.*

- Main Patient exclusion criteria
  1. Immunotherapy in the past 5 years with allergenic extract of *Cupressus arizonica* or other allergenic extracts that may interfere with the allergen to be tested due to a high degree of cross-reactivity (especially with *Cupressus sempervirens* or other *Cupressaceae* of the genera *Cupressus*

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and *Juniperus)* .

2. Use of drugs that may interfere with the skin reactions before or during the study (e.g., antihistamines).
3. Treatment with any of the following medications: tricyclic or tetracyclic antidepressants, or MAOIs, phenothiazines, beta-blockers, chronic use of oral corticosteroids, or oral or parenteral corticosteroid use in repeated and intermittent regimens (> 10 mg / day of prednisone or equivalent).
4. Pregnancy or lactation.
5. Dermographism affecting the skin area at the test site at either study visit.
6. Atopic dermatitis affecting the skin area at the test site at either study visit.
7. Urticaria affecting the skin area at the test site at either study visit.
8. Participation in another clinical trial within the last month.
9. Patients suffering from severe conditions (heart failure, severe or uncontrolled respiratory diseases, endocrine diseases, clinically relevant liver or kidney diseases or hematological diseases).
10. Patients suffering from diseases of the immune system clinically relevant, both autoimmune and immune deficiencies.
11. Patients suffering from diseases or disorders that limit the use of adrenaline (coronary artery disease, severe HBP, etc.).
12. Serious psychiatric, psychological or neurological disorders.
13. Abuse of alcohol, drugs or medication in the previous year.
14. Patients that have received anti-IgE treatment (omalizumab).

**Test product, dose and mode of administration, batch number:**

*Cupressus arizonic*a allergen extract, vial 1: 15 mg/ml. Batch nº: H-15 *(Expiry date:* 11/2015*)*.

*Cupressus arizonic*a allergen extract, vial 2: 10 mg/ml. Batch nº: H-14 *(Expiry date:* 11/2015*)*.

*Cupressus arizonic*a allergen extract, vial 3: 5 mg/ml. It was obtained by diluting vial 1.

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*Cupressus arizonic*a allergen extract, vial 4: 1 mg/ml. It was obtained by diluting vial 2.

Solvent SC-1: Batch no: H-11 (*Expiry Date: 02/2016).*

Solvent SC-2: Batch no: H-12 (*Expiry Date: 02/2016).*

Negative control: Batch no.: 22265 (*Expiry Date*: 09/2016).

Positive control (Histamine dihydrochloride 10 mg/ml): Batch no.: 2628214 (*Expiry Date:* 02/2016*).*

The investigational medicinal products were applied on the skin by the prick test method:

The investigator or designee placed a drop on the patient´s volar side of the forearm (in duplicate) of the positive control, the negative control and the 4 concentrations of *Cupressus arizonic*a allergen extract. Prick tests were placed in areas 5 cm from the wrist or 3 cm from the antecubital fossae. In addition, test drops were allocated at least 3 cm apart to avoid wheal interferences.

For the Titrated Skin Prick test response evaluation, test sites were inspected and the responses recorded 15-20 min after application. The contours of the wheals (and not the erythema) were outlined with a skin marking pen, ensuring no red skin around the wheal was inside the encircled area. Using translucent tape (pressing it gently against the wheal), the contours were transferred into the corresponding page of the Case Report Form.

**Duration of study:** The clinical trial consisted of 1 or 2 site visits per patient of approximately 60 minutes, depending on the possibility to assess eligibility criteria during visit 1.

**Criteria for evaluation:** Wheal size area ($mm^2$) on the skin at the site of the puncture during the immediate phase.

**Statistical methods:**

The wheal areas ($mm^2$) resulting on the skin as a consequence of the exposure to *Cupressus arizonic*a allergen extract at different concentrations, positive and negative controls, by the Prick-test procedure were measured.

For every patient, the geometric means of the wheal areas provoked by the *Cupressus arizonic*a allergen extract and the histamine were calculated. A logarithmic transformation of them were performed subsequently

For each patient, a linear regression analysis, using the method of least squares, was performed. Then it was

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checked if the patients fulfilled the Nordic Guidelines (1) criteria with slight modifications, for analysis.

For each valid patient, the value of the geometric mean of the wheal area provoked by histamine (10 mg/ml) was inserted into the equation to derive the corresponding concentration of the *Cupressus arizonic*a allergen extract that elicited a wheal size equivalent to that of histamine (10 mg/ml). The median of the concentrations of the *Cupressus arizonic*a allergen extract that elicits a wheal size equivalent to that of histamine (10 mg/ml) of the valid patients corresponds to 10 HEP/ml. This value indicates the *in vivo* biological activity of the *Cupressus arizonic*a allergen extract.

**Safety results:** No adverse events were reported in the clinical trial.

**Conclusion:** The biological activity of the *Cupressus arizonic*a allergen extract equivalent to 10 HEP/mL is obtained using **1.92 mg/mL** of this allergen. The administration of the study medication by prick testing was well tolerated and safe.

**Date of report: 6/AUG/18**