Clinical trial results:

Biological standarization of allergic extracts of wild grasses: *Phleum pratense, Lolium perenne, Poa pratensis y Dactylis glomerata* and its mixture to determinate their biological activity, the In-House Reference Preparation, measures in histamine unit equivalents (HEP) in sensitized patients

Summary

EudraCT number	2019-002644-24
Trial protocol	ES
Global end of trial date	February 2021

Results information

Result version number	V1 (current)
This version publication date	December 2024
First version publication date	December 2024

Trial information

Trial Identification

Sponsor protocol code	DIA-STA-11-01-19

Additional study

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	-
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	DIATER Laboratorio de Diagnóstico y Aplicaciones Terapéuticas, S.A.
Sponsor organisation address	Avda. Gregorio Peces Barba 2, Madrid, Spain, 28919
Public contact	Medical Dept. DIATER Laboratorio de Diagnóstico y Aplicaciones Terapéuticas, S.A., 0034 914966013, departamento.medico@diater.com
Científic contact	Medical Dept. DIATER Laboratorio de Diagnóstico y Aplicaciones Terapéuticas, S.A., 0034 914966013, departamento.medico@diater.com

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)?	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	February 2022
Is this the analysis of the primary completion data?	Yes
Primary completion date	February 2022
Global end of trial reached?	Yes
Global end of trial date	February 2021
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

The primary objective of this study was to evaluate in vivo the concentration of the allergenic extracts of wild grass pollen individually: *Phleum pratense, Lolium perenne, Poa pratensis and Dactylis glomerata* and their mixture that provoked a papule of a size equivalent to that produced by a solution of histamine dihydrochloride at 10 mg/ml. And standardize the allergenic extracts in biological units.

Protection of trial subjects:

Each potential subject was adequately informed of the aims, method, anticipated benefits and potential hazards of the study and the discomfort that it might entail. All of them were informed that they were free to participate in the study and stop their participation at any time. Participants had the opportunity to make all kind of questions about the study, and every subject confirmed his or her participation by filling in and signing the informed consent form. Written informed consent was obtained from each subject prior to the performance of any study-specific procedures.

Background therapy: NA

Evidence for comparator:-

Actual start date of recruitment	06 October 2020
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjests enrolled per country

Country: Number of subjects enrolled	Spain: 31
Worldwide total number of subjects	31
EEA total number of subjects	31

Notes:

Subjects enrolled per age group

In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	31
From 65 to 84 years	0
85 years and over	0

Sujects disposition

Recruitment

Recruitment details:

Recruitment period (from the date of the first site ready to recruit to the date for the last patient entered into the study): October 2020 to February 2021. FPFV: 6 October 2020; LPLV: 18 February 2021
31 patients were enrolled.

Pre-assignment

The patients were allocated to the the following arm:

EXPERIMENTAL GROUP:
31 patients who had been diagnosed positive for any of the allergens of the study by SPT Specific IgE positive received the allergenic extracts of wild grass pollen individually: *Phleum pratense, Lolium perenne, Poa pratensis and Dactylis glomerata* and their mixture.

Period 1

Period 1 title	Overall trial (overall period)
Is this the baseline period?	Yes
Allocation method	controlled
Blinding used	Not applicable

Arms

Are arms mutually exclusive?	NA
Arm title	Treatment arm

Arm description:
Patients who had been diagnosed positive for any of the allergens of the study by SPT Specific IgE positive

Arm title	Experimental group
Investigational medicinal product name	*Phleum pratense, Lolium perenne, Poa pratensis y Dactylis glomerata* and its mixture
Investigational medicinal product code	V04CL
Other name	NA
Pharmaceutical forms	Solution for injection / skin-prick-test
Routes of administration	Cutaneous use

Dosage and administration details:

The test consisted the direct application of the four concentrations of the experimental allergenic extract (10 mg/ml; 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml), together with the prick mixture of grasses (10 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml), the positive control of histamine and the negative control of saline solution each allergen upon the skin and the performance of a Prick test.

Baseline characteristics

Reporting groups

Reporting group title	Overall trial

Reporting group description:

Patients who signed informed consent and with complete data.

Reporting group values	Experimental group
Number of subjects	31 (100%)
Age categorical Units: Sujects	
Adults (18-64 years)	31 (100%)
Gender categorical Units: Subjects	
Female	18 (58%)
Male	13 (42%)

Subject analysis sets

Subject analysis set title	PP population
Subject analysis set type	Per protocol

Subject analysis set description:

Patients who signed informed consent and with complete data

Subject analysis set title	ITT Population
Subject analysis set type	

Subject analysis set description:

Patients who signed informed consent.

Reporting group values	ITT Population	PP population
Number of subjects **[1]**	31	
Phleum pratense	31	26
Lolium perenne	31	21
Poa pratensis	31	26
Dactylis glomerata	31	26

Note:

[1] The patients who were excluded because of they did not meet the statistical criteria for analysis of the Nordic Guidelines:

- r ≥ 0.85 (where r is the correlation coefficient).
- Ml > mH = MI is the geometric mean using the two areas of the papules produced by the four concentrations of the individual allergenic extract of wild-type grasses or their mixture and mH the histamine papule (10 mg/ml).

Lolium perenne	***Phleum pratense***	***Poa pratensis***	***Dactylis glomerata***	**Mezcla de las gramíneas**
0106 - Ml < mH	**0115** - Ml < mH	**0107** - r < 0,85	**0106** - r < 0,85	**0110** - r < 0,85
0107 - r < 0,85	**0117** - r < 0,85	**0115** - Ml < mH	**0109** - r < 0,85	**0112** - r < 0,85
0109 - r < 0,85	**0118** - r < 0,85	**0116** - r < 0,85	**0117** - r < 0,85	**0117** - Ml < mH
0111 - Ml < mH	**0123** - r < 0,85	**0119** - r < 0,85	**0131** - r < 0,85	**0120** - r < 0,85
0117 - Ml < mH	**0131** - r < 0,85	**0126** - r < 0,85		**0128** - r < 0,85
0120 - Ml < mH				
0123 - Ml < mH				
0125 - r < 0,85				
0129 - Ml < mH				
0131 - Ml < mH				

End points

End points reporting groups

Reporting group title	Experimental group

Reporting group description:
Patients who had been diagnosed positive for any of the allergens of the study by SPT Specific IgE positive

Primary: To evaluate in vivo the concentration of allergic extracts of wild grasses individually and its mixture

End point title	To evaluate in vivo the concentration of allergic extracts of wild grasses individually: *Phleum pratense, Lolium perenne, Poa pratensis y Dactylis glomerata* and its mixture. And standardize the allergenic extracts in biological units

End point description:

The Biological standarization consisted the direct application of the four concentrations of the experimental allergenic extracts (10 mg/ml; 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml), together with the prick mixture of grasses (10 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml), the positive control of histamine and the negative control of saline solution upon the skin and the performance of a Prick test. Where the Prick test gave a positive response, the areas of erythema and papules, were measured (mm^2).
The skin tests were used to obtain the optimum allergen extract concentration that provokes a response with the same wheal area to that obtained with the positive control solution of histamine dihydrochloride 10 mg/mL reaction.

The conclusion was that the biological activity of the study allergens and thir mixture equivalent to 1 HEP/mL was:
0.096 mg/mL for Lolium perenne; 0.197 mg/mL for Phleum pratense; 0.156 mg/mL for Poa pratensis; 0.200 mg/mL for Dactylis glomerata and 0.195 mg/mL for the mixture of grasses.

End point type	Primary

End point timeframe
1 day

Statistical analyses

Statistical analysis title	Outcome 1

Statistical analysis description: Validated and protected Microsoft Excel specifically designed for these analyses

Comparison groups	NA
Number of subjects included in analysis	31
Analysis specification	Pre-specified
Analysis type	
P-value	>0.05
Method	Based on the Nordic Guidelines
Parameter estimate	Geometric mean difference (final values) **[1]**
Confidence interval	
Level	95 %
Sides	
Upper limit	0.85
Variability estimate	standard deviation

Note:
[1] The geometric mean of the areas of the papules (mm2) of each patient was calculated for each of the dilution series of each extract, as well as that of the positive control solution of histamine dihydrochloride 10 mg/mL. For each subject, a linear regression analysis was performed using the least squares method. The linear regression model plotted the logarithm of the geometric mean of the papules elicited by all concentrations of each allergenic extract in the test against the logarithmic transformation of these concentrations.
Subjects were considered valid subjects for analysis if they met the following criteria according to the Nordic Guidelines.
For each valid subject, the geometric mean value of the area of papules provoked by histamine (10 mg/ml) was also entered into the equation to obtain the corresponding concentration of the allergen extract provoking a skin reaction of a size equivalent to or larger than that produced by histamine (10 mg/ml). The median value of the histamine-equivalent study drug concentration obtained for all valid patients represents the concentration corresponding to 1 HEP or 10,000 Biological Units (BU)/mL. This value indicates the in vivo biological activity of the allergenic extracts of Phleum pratense, Lolium perenne, Poa pratensis and Dactylis glomerata and their mixture.

Adverse events

Adverse events information

Timeframe for reporting adverse events:

From enrollment to 1 week after completion of the skin tests

Assessment type	Systematic

Dictionary used

Dictionary name	MedDRA
Dictionary version	

Reporting groups

Reporting group title	

Reporting group description:

No treatment-related adverse events were reported during this study.

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? No

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

None reported