Trials

STUDY PROTOCOL

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Efficacy and safety of a house dust mites allergoid in patients with allergic rhinitis—PROACAROS study: protocol for a randomized controlled trial

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Abstract

Background There is an important heterogeneity of the clinical research done to date for allergen immunotherapy (AIT). We plan to assess the safety and efficacy of a house dust mite (HDM) polymerized allergen extract mixture for allergic rhinoconjunctivitis (AR) according to both the EMA and European Academy of Allergy and Clinical Immunology (EAACI) guidelines for the clinical development of products for the treatment of AR.

Methods We will perform a double-blind, placebo-controlled, randomized parallel group phase III clinical trial to assess the clinical efficacy and safety of a polymerized *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* allergen extract mixture (Beltavac®) to treat perennial AR in children and adults. Patients with moderate or severe rhinitis symptoms, either associated or not with asthma and confirmed HDM sensitization and without relevant concomitant conditions that may interfere with the planned evaluations test are eligible. Patients will be randomized in a 1:1 ratio to either the active AIT or placebo. The experimental group will receive 12 monthly AIT doses via subcutaneous route with a potency of 2 RC/ml per allergen. The expected sample size is 250 patients from 16 sites in Spain. The main efficacy outcome is the Combined Symptom and Medication Score (CSMS) for rhinitis. It will be patients' self-assessed and collected through a phone App developed ad hoc for the study to improve the patient adherence and the quality of data. Main secondary outcomes include expanded CSMS for rhinoconjunctivitis symptoms, control of rhinitis, specific IgE and IgG_4 values, quality of life, and the number of adverse reactions. Health-related direct and indirect costs will be also evaluated. Finally, several exploratory parameters will be used to assess the severity of asthma.

Discussion This phase III clinical trial will be of interest to contribute to the scientific evidence about the efficacy and safety of AIT with allergoids. Our working hypothesis is that the investigational product in patients with AR associated or not with asthma is superior to placebo in providing a clinically significant improvement according to the standards defined by the EAACI. This trial will also supply valuable information about patients reported outcomes using health technology for rhinoconjunctivitis and asthma assessment.

Trial registration EudraCT 2018–003427-11. Date on which this record was first entered in the: 2021–06-14.

Keywords Immunologic desensitization, Dust mite allergy, Health economics, Vaccines

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Administrative information

Title {1}	Efficacy and Safety of a House Dust Mites Allergoid in Patients with Allergic Rhinitis – PROACAROS Study: Protocol for a Randomized Controlled Trial
Trial registration {2a and 2b}	EudraCT: 2018–003427-11 Registration in the EU Clinical Trials Register: https://www.clinicaltrialsregister.eu/ctr-search/trial/2018–003427-11/ES
Protocol version {3}	Version 4.0 (July 1 st, 2022)
Funding {4}	This study is funded by Probelte Pharma S.L.U. and CDTI funds
Author details {5a}	Inmaculada Buendia-Jimenez (IBJ) and Maria Matas-Ros (MMR), Medical Affairs Department, Probelte Pharma (Murcia, Spain); Teresa Garriga-Baraut (TGB), Pediatric Allergy Unit, Vall d'Hebron Universitary Hospital, Barcelona (Spain); Albert Rogers-Reig (ARR) Allergy Section. Germans Trias Pujol Universitary Hospital, Badalona (Spain); Ana I. Tabar- Purroy (ATP) Allergy Department. Navarra Universitary Hospital, Pamplona (Spain) IBJ and ATP are responsible for the conception of the study, protocol design and study coordination. MMR is responsible for the study management and monitorization of the study. TGB and ARR contribute to the protocol design and recruitment of the study
Name and contact information for the trial sponsor {5b}	Inmaculada Buendía Jiménez, Probelte Pharma S.L.U.; Telephone: + 34 608 933 618; email: inmaculadabuendia@probeltepharma.es
Role of sponsor {5c}	The sponsor will be involved in study design, provide the investigational medicinal product and the randomization sequence, handle adverse events' notifications to the corresponding regulatory authorities, and develop annual drug safety update reports The sponsor will also ensure and maintain the quality and control systems throughout the study and develop the clinical monitoring plan The sponsor will not be involved in the collection, management, analysis and interpretation of data

Introduction

Background and rationale {6a}

Subcutaneous AIT with native extracts dates back almost a hundred years and remains a healthcare centre-administered treatment, despite all medical advances. This implied weekly/monthly hospital visits (depending on the treatment stage) for at least 3 years—and a minimum 30-min observation period after each dose. Adverse reactions are also common, either locally (26–86%) or systemically (2–5%); severity is variable, and anaphylactic shocks have been reported [1, 2]. Unsurprisingly, treatment adherence is an issue, being absenteeism the leading cause of discontinuity.

To reduce allergenicity whilst maintaining immunogenicity, glutaraldehyde-polymerized allergen extracts (allergoids) were developed, proving to be safer than unmodified vaccines whilst retaining clinical efficacy [3–5]. The safety improvement allowed the development of rush administration regimens, implying single monthly administrations since the beginning of the treatment [6]. This reduces the required number of visits to healthcare centres, improves treatment efficacy and adherence, and decreases healthcare costs [6].

Beltavac® is an HDM polymerized allergen extract mixture (*Dermatophagoides pteronyssinus* [DPT] + *Dermatophagoides farinae* [DF]) that has been manufactured and distributed in Europe for almost 20 years. Previous clinical studies conducted with this product [7–9] as well as existing literature on subcutaneous immunotherapy with polymerized extracts support its safety and effectiveness [10]. Since there was considerable heterogeneity in the clinical research methodology used in the evaluation of AIT, the European Academy of Allergy and Clinical Immunology (EAACI) published recommendations for conducting clinical trials in this area [11]. The objective of this clinical trial that was designed following the latest EAACI recommendations is to assess the safety and efficacy of Beltavac® in AR patients (associated or not with asthma), and provide scientific evidence on the general safety and efficacy of the HDM allergen immunotherapy.

Objectives {7}

Our hypothesis is that subcutaneous rush immunotherapy with a DPT/DF mite polymerized allergen extract mixture (Beltavac® DPT/DF) for 12 months is superior to placebo in producing a clinically significant improvement in patients with allergic rhinitis/rhinoconjunctivitis (associated or not with asthma).

The primary objective is to assess the efficacy of the subcutaneous rush immunotherapy with Beltavac® for 12 months for treating HDM-sensitized allergic rhinitis.

Secondary objectives are:

(1) To assess the efficacy of Beltavac® in controlling rhinoconjunctivitis.

(2) To assess its global efficacy on allergic symptoms.
(3) To assess its safety.
(4) To assess its effect on patients' quality of life.
(5) To assess its effect on the serum levels of specific immunoglobulins.
(6) To perform an economic assessment of its clinical effect.

Other exploratory objectives are:

(1) To assess its efficacy in treating HDM-sensitized allergic asthma.
(2) To assess its efficacy in controlling allergic asthma.

Trial design {8}

This study will consist of a multicentre, phase III, double-blind, parallel-group, placebo-controlled, randomized clinical trial. Given the variability in individual clinical responses, unpredictability and allergen exposure variability, and the subjective nature of symptom assessment, we chose a superiority design to enhance trial sensitivity.

Methods: participants, interventions and outcomes

Study settings {9}

The patients will be recruited in the allergy units of hospitals and allergy clinics distributed throughout Spain. An updated detailed list can be found in: https://reec.aemps.es/reec/estudio/2018–003427-11.

Eligibility criteria {10}

Patients meeting all inclusion criteria and none of the exclusion criteria will be eligible for this trial.

There are two sets of inclusion criteria, one for the screening visit and another for the randomization visit that is scheduled 4–6 weeks later.

Inclusion criteria at the screening visit

- Patients aged 12–65 years old, of either sex.
- With moderate or severe rhinitis symptoms (as defined by the Allergic Rhinitis and its Impact on Asthma [ARIA] guideline [12]) associated or not with well- or partially controlled asthma (according to the Spanish Guideline for Asthma Management 5.0 [GEMA 5.0] [13]).
- Confirmed DPT or DF sensitization by a positive Prick test (mean papule diameter ≥ 3 mm) with a commercial standardized allergen extract, and a class three or higher serum extract-specific IgE value (> 3.5 kU/L) within 6 months of study start.
- In case of asthmatic patients, an Asthma Control Test (ACT) score > 19.
- Women of childbearing potential under highly effective contraceptive measures for at least 1 month prior to the screening visit and commit to continue using them during the study period.
- Patients who sign the written informed consent.

Inclusion criteria at the randomization visit

- Negative pregnancy test.
- A Combined Symptom-Medication Score for nasal symptoms (CSMS4) ≥ 1.5 taken from patients' diaries completed during the screening period.
- A Peak Expiratory Flow (PEF) > 80% of the patient's best personal value (in case of asthmatic patients).
- Patients able to fulfill the electronic patient diaries during the screening period.

Exclusion criteria

- Patients concomitantly sensitized to other allergens besides HDM in whom the recruiting investigator expects clinically relevant symptoms to develop that may interfere with the study evaluation periods.
- Patients receiving non-HDM allergens immunotherapy during the study period.
- Patients with poorly controlled or severe asthma (i.e. patients requiring tier 5–6 therapeutic approaches, according to the GEMA 5.0 guideline).
- Autoimmune diseases or immunodeficiency.
- Malignant neoplasms, severe cardiovascular diseases, severe mental illnesses, or other relevant chronic diseases that may interfere with the study results.
- Past medical history of anaphylaxis with cardiorespiratory symptoms.
- Use of immunosuppressive medication (e.g. cyclosporine, azathioprine, omalizumab) from 6 months before screening until the end of the study.
- Treatment with beta-blockers during the study.
- Immunotherapy recipients (with HDM or other allergenic extracts) with a failed response in the last 5 years.
- Hypersensitivity to any component of the study treatment.
- Patients receiving any other vaccine 1 week before treatment start or waiting for the second dose of the anti-SARS-CoV-2 vaccine.
- Pregnant or lactating patients.

Who will take informed consent? {26a}

Investigators from each participating centre will be responsible for obtaining informed consent before starting any trial-related procedure. They will handle patients the information sheet (containing detailed yet comprehensible information on all study-related procedures, clinical data collection, study product, and the potential risks and benefits associated) and written informed consent forms. Additionally, they will provide detailed oral explanations and answer any doubts that may arise. The patients will have time to review the information and consent sheets and will be able to ask the necessary questions before signing. They will also be informed that their participation is voluntary and that they will be able to leave the study at any time without any prejudice.

Underage patients will receive an information sheet and written informed consent adapted to their understanding ability, whilst their parents/legal representatives will receive these documents' regular versions.

All information sheets and written informed consent versions will undergo Institutional Review Board (IRB) evaluation. The investigators will keep copies of all the signed consent forms in the patients' medical records.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

The patient information sheet will contain full explanations about how biological samples will be obtained, stored, and used. Before participating, patients will also have to sign a specific consent form for the collection and processing of blood samples and associated data for its use in future investigations in the area of AIT.

Interventions

Explanation for the choice of comparators {6b}

The placebo effect in immunotherapy trials is considerable, varying from 24 to 33% during the first year of treatment [14]. Additionally, local allergic adverse events (AEs) are frequent with specific immunotherapy [15]. Hence, placebo was chosen as comparator.

Placebo will consist of a suspension for subcutaneous injection with the same components of the investigational medicinal product (IMP) but without the allergenic extract. These ingredients will be aluminium hydroxide gel (adjuvant), sodium chloride, phenol (preservative), and water for injection.

Intervention description {11a}

The IMP will consist of a glutaraldehyde-polymerized HDM allergen extract mixture of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Beltavac®) administered subcutaneously. Previously, the allergenic extract undergo in vivo and in vitro standardization following the Nordic method [16]—the allergen concentration that induces a 10 mg/mL histamine-like cutaneous response is equivalent to 1 RC/mL.

The study products will be administered at the allergy units of the participating healthcare centres. All procedures will be carried out by trained healthcare professionals in a fit-for-purpose location. The potency will be of 2 RC/mL for each allergen extract, which has proven safety and effectiveness profiles in routine clinical practice. The quantification of major allergens is 15 µg/mL for *Der p*1, 12 µg/mL for *Der p*2, 22 µg/mL for *Der f*1, and 17 µg/mL for *Der f*2, all within the effective dose range recommended for mite immunotherapy [16].

Dose schemes are shown in Table 1. Patients will be initially administered two doses with a 30-min interval between them: (i) an initial 0.2-mL dose will be administered in one arm; (ii) a 0.3-mL dose will be administered 30 min later in the other arm. Thereafter, subsequent 0.5-mL doses will be administered every 30 (+ 15) days for 12 months. Patients will remain under close medical supervision for at least 30 min after treatment administration.

Criteria for discontinuing or modifying allocated interventions {11b}

Immunotherapy administration will be delayed in case of (i) upper respiratory tract infection with fever (treatment will be administered after 7 days without fever); (ii) asthmatic exacerbation (wait for 24 h after resolution); (iii) severe skin condition (wait until resolution); (iv) severe intercurrent illness (wait for stabilization); (v) worsening of the allergic process (wait for stabilization).

If the treatment is interrupted for more than 8 weeks, patients will return to the starting dose scheme (0.2 + 0.3 mL) and then return to the 0.5 mL dose the following month. If the treatment interruption lasts more than 12 weeks, participants may be withdrawn from the trial.

Strategies to improve adherence to interventions {11c}

Participants will assess their symptoms and medication usage through a self-complete electronic diary during

Table 1 Allergen doses administered in each visit

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Month	1	2	3	4	5	6	7	8	9	10	11	12
Dose (mL)	0.2 + 0.3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

V visit

4-week periods before visits V1, V7, V10, and V13. These self-assessment activities will be carried out through an electronic diary (accessible online through the patient's mobile phone). For this purpose, a validated phone application has been developed ad hoc for the study and is scheduled to send reminders to signal when to start completing the diaries and to make patients aware of outstanding tasks and upcoming visits, and will encourage them to continue participating. Asthmatic patients will have also access to another application that will allow them to monitor their lung function (by measuring patients' PEF) through a device connected to the phone. The investigators will have instantaneous access to the data recorded through the phone applications so that he/she can contact the patients in case of noncompliance and take the appropriate measures.

Relevant concomitant care permitted or prohibited during the trial {11d}

The concomitant administration of immunosuppressive treatments, beta-blockers, and any other immunotherapy besides Beltavac® is prohibited during this trial. All concomitant medications taken (with or without a prescription) must be reported in the electronic Case Report Form (eCRF). Other vaccines are authorized, but a 2-week interval between the vaccine and Beltavac® administration is mandatory.

Symptomatic treatment for allergic rhinitis/conjunctivitis and allergic asthma is also allowed during the trial, but restricted to agents at doses stipulated in the protocol ((i) as first step, levocabastine/azelastine/levocetirizine for rhinitis and inhaled budesonide/fluticasone at low doses for asthma, (ii) as second step, nasal topical fluticasone for rhinitis and inhaled budesonide/fluticasone at low doses associated with salmeterol/formoterol/vilanterol for asthma, and (iii) as third step, oral methylprednisolone for rhinitis and inhaled budesonide at medium doses plus salmeterol/formoterol/vilanterol for asthma, see more details on Fig. 1). If necessary, patients may additionally use a short-acting inhaled β2 adrenergic agonist (SABA: salbutamol or terbutaline) as rescue therapy on demand. This medication will not be provided by participating sites and must be acquired by the patients in pharmacies.

Provisions for post-trial care {30}

Participants will be offered an additional year of treatment free of charge with the study product after trial completion.

Outcomes {12}

Following the EAACI recommendation [11], our primary outcome measure is the CSMS4 (Fig. 1). Participants will assess their nasal symptoms and medication usage through a self-complete electronic diary during 4 weeks period before V1, V7, V10, and V13. The number of items, completion period, scaling, and scoring will be the same as in the cited recommendation. This outcome measure has not yet been fully validated, and there is scarce data on the expected effect sizes, for which a conservative approach has been used for calculating the sample size (see 'Sample size{14}'). In addition, we plan to take advantage of the study data to assess the measurement properties of the CSMS and contribute to its clinical validation (see the 'Discussion'). Furthermore, this score and its components will also be used as secondary outcome measures, as shown in Table 2. Secondary outcomes will include specific tools to measure health-related quality of life (mini Rhinoconjunctivitis Quality of Life Questionnaire, RQLQ) and rhinitis symptoms (Rhinitis Control Assessment Test, RCAT). Table 2 provides a full description of all outcome measures and the rationale for using them in the study.

Participant timeline {13}

Please refer to Table 3.

Sample size {14}

The sample size has been initially calculated considering (i) the primary measure of efficacy (a score ranging from 0 [best health status] to 6 points [worse health status]); (ii) the statistic test chosen (Wald t-test) to estimate the (fixed) treatment effect in a mixed general linear model for repeated measures of a Gaussian continuous variable; (iii) a superiority hypothesis; (iv) the four timepoints where the main efficacy measure will be assessed; (v) a moderate autocorrelation between repeated measures; (vi) a nominal significance level of 5% (two-sided); (vii) 80% statistical power; and (viii) a forecasted 20% dropout rate. Notably, the scarce data available on the expected effect size for the main efficacy measure (CSMS4) is a limitation, and thus a conservative (lower level) effect size was adopted based on the available results [10, 17] (active treatment − placebo Standardized Mean Difference [SMD] ≈ 35%). Under these conditions, 71 patients per group would be required to detect a between-group mean difference of 0.35 (in absolute values) for the main efficacy measure and a common standard deviation (SD) of 1.00 (corresponding to a 35% SMD), considering that the autocorrelation is 0.4. To compensate for the forecasted 20% dropout rate, a total of 178 patients would be required.

However, CSMS are unlikely to follow a normal distribution but rather more closely resemble count data. Therefore, analyses could be performed using generalized estimating equations for count data (i.e. the

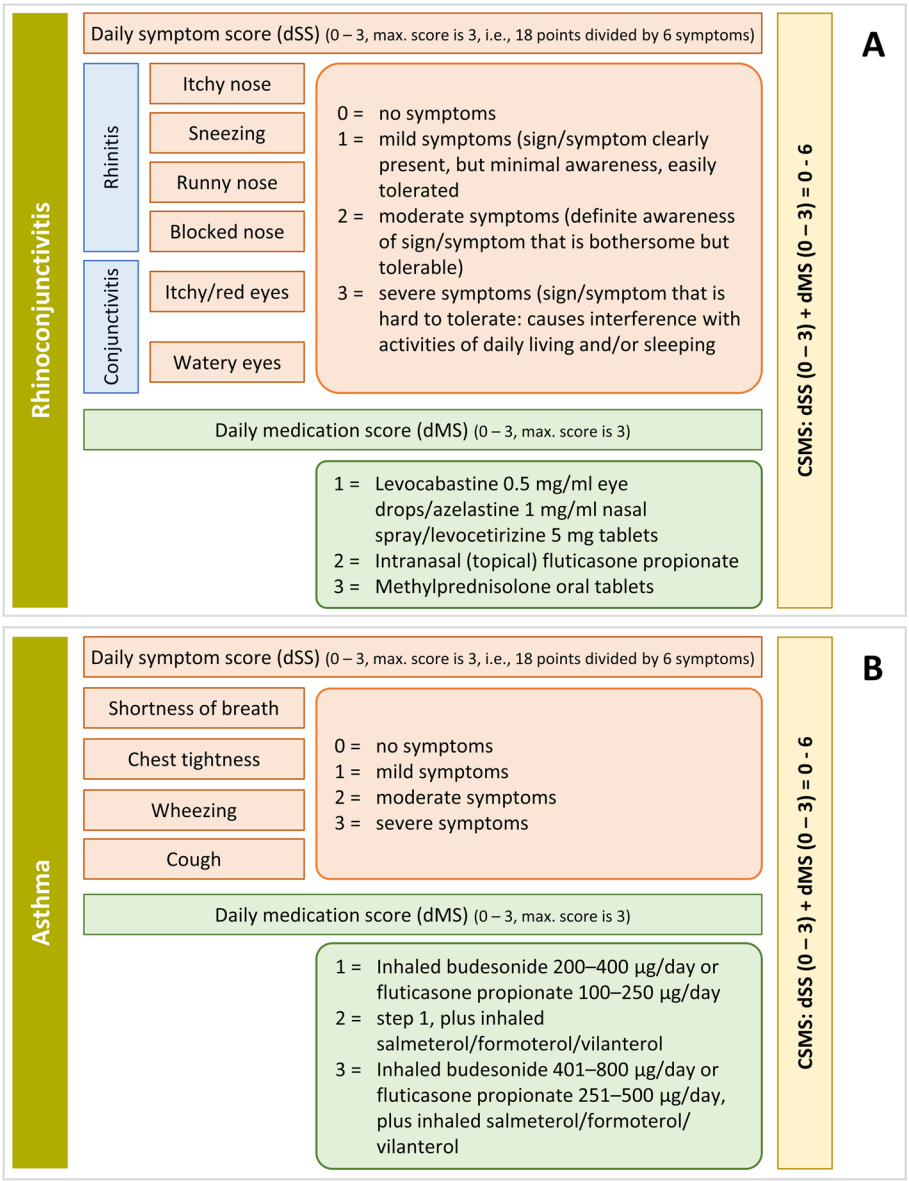

Fig. 1 Combined symptom and medication scale for rhinoconjunctivitis (**A**) and asthma (**B**). CSMS: Combined symptom medication scale

Poisson error distribution). Considering time-averaged difference tests using Generalized Estimating Equations (GEE) for repeated count data measurements, a first-order autoregressive structure for the covariance matrix, and the aforementioned specifications, sample sizes ranging between 129 and 386 patients are obtained depending on the expected score at the end of the study in the placebo group (129 patients with 0.81 and 386 patients with 2.81). Based on the work by Bozek et al. [23] that reported a score of 1.82 in the placebo group, the required sample would be 257 patients. Hence, the recruitment target for this trial is set 250 patients.

Sample size calculations were performed with the PASS software, version 16.0.1 (PASS 16 Power Analysis and Sample Size Software [2018], NCSS, LLC, Kaysville, Utah, USA, https://www.ncss.com/software/pass/).

Recruitment {15}

Participant recruitment will take place during 24 months in different participating healthcare centres (see 'Study Settings {9}'). Each month, the study investigators will

Table 2 Primary and secondary outcome measures

Outcomes	Justification
Primary outcome measure[a]	
1) Mean (SD) CSMS4 in visits V1, V7, V10, and V13	This outcome will be used as recommended by the EAACI [17]. Since the primary objective is to assess the efficacy of Beltavac® on HDM-sensitized allergic *rhinitis*, we will calculate the dSS for the primary outcome measure based only on the four nasal symptoms (dSS4)—i.e. without the eye symptoms. Symptom scores vary from 0 to 3 (the higher, the worse). Medication intake is also assessed by a score ranging from 0 to 3, where no medication intake yields a score of zero, and each step of the maintenance treatment adds one point. The combined score ranges from 0 to 6 (sum of daily symptom and medication scores)
Secondary outcome measures[a]	
1) Mean (SD) dSS4 in visits V1, V7, V10, and V13	As recommended by the EAACI, the dSS 'alone' may be used as a secondary parameter, as it does not account for any concomitant medication use. We will assess the dSS4 (only nasal symptoms) and dSS6 (nasal + eye symptoms) separately
2) Mean (SD) dSS6 in visits V1, V7, V10, and V13	
3) Mean (SD) dMS in visits V1, V7, V10, and V13	Following the EAACI recommendation, we will use the dMS to measure rescue medication usage
4) Mean (SD) CSMS in visits V1, V7, V10, and V13	Global CSMS assessment es recommended by the EAACI (considering nasal + eye symptoms)
5) Percentage of days without symptoms and without rescue medication in visits V1, V7, V10, and V13	As a measure of well and severe days, according to the EAACI recommendation
6) Mean (SD) VAS score in visits V1, V7, and V13	The VAS is fully validated in adult patients [18] and correlates well with the severity of allergic rhinitis [28]. Both participants and investigators will complete it in visits V1, V7, and V13
7) Mean (SD) RCAT score in visits V1, V7, and V13	The RCAT is a patient-based questionnaire widely used to evaluate rhinitis control [19]. Participants will complete it in visits V1, V7, and V13
8) Mean (SD) mini-RQLQ score in visits V1, V7, and V13	The RQLQ and the mini-RQLQ are validated tools capable of distinguishing severe, controlled and uncontrolled patients from healthy subjects [17]. The mini-RQLQ seems to be more responsive to change than the classical version [20]. Participants will complete it in visits V1, V7, and V13
9) Mean (SD) serum concentrations of total IgE and IgG4 specific to DPT and DF, *Der p1, Der p2, Der p23, Der f1*, and *Der f2* in the screening visit and visits V7 and V13	Biomarkers of the AIT-induced immune response
10) Number (percentage) of local and systemic AEs in 12 months	AEs assessment will be performed following the WAO grading system [21]
11) Mean (SD) biochemical and haematological parameters' values in the screening and V13 visits	As an additional safety monitoring measure
12) Mean (SD) in-hospital days and ICU admissions in 12 months	For direct and indirect disease cost assessment
13) Mean (SD) number of visits to the ER, GP and specialist physician in 12 months	
14) Mean (SD) work/school days lost in 12 months	
15) Mean (SD) medication intake in 12 months	
Exploratory outcome measures[a]	
1) Mean (SD) Combined Asthma Symptoms and Medication score in visits V1, V7, V10 and V13	This combined scale was created based on the GEMA 5.0 guideline [13] and assesses daily the severity of allergic asthma symptoms and medication requirements, similarly to the CSMS
2) Mean (SD) Asthma Symptom score in visits V1, V7, V10 and V13	
3) Mean (SD) Asthma Medication score in visits V1, V7, V10, and V13	
4) Mean (SD) SABA inhalations/day required for symptom relief in visits V1, V7, V10, and V13	Asthmatic participants will be asked to register their daily SABA inhalations as an additional measurement of disease control
5) Mean (SD) PEF volume in visits V1, V7, V10, and V13	Asthmatic participants will self-perform the spirometry through a digital PEF metre connected to their smartphones (Android or iOS). They will perform these measurements at visit V1 and thereafter daily for 4-week periods before visits V7, V10, and V13, as an additional measurement of disease control
6) Mean (SD) asthma exacerbation episodes in visits V1, V7, V10, and V13	Asthmatic participants will be asked to register the number of exacerbation episodes during their participation in the study as an additional measurement of disease control
7) Mean (SD) ACT score in visits V1, V7, and V13	The ACT assesses the control of asthma symptoms over the past 4 weeks [22]. Asthmatic patients will complete it in visits V1, V7, and V13

SD standard deviation, *CSMS4* Combined Symptom and Medication Score – 4 nasal symptoms, *HDM* House Dust Mites, *dSS4* daily Symptom Score – 4 nasal symptoms, *dMS* daily Medication Score, *dSS6* full daily Symptom Score (nasal + eye symptoms), *CSMS* full Combined Symptom and Medication Score (nasal + eye symptoms), *EAACI* European Academy of Allergy and Clinical Immunology, *VAS* visual analog scale, *V* visit, *RCAT* Rhinitis Control Assessment Test, *Mini-RQLQ* Mini Rhinitis Quality of Life Questionnaire, *GEMA* Spanish Guideline for Asthma Management (*Guía Española para el Manejo del Asma*), *SABA* Short-Acting Beta Agonist, *PEF* Peak Expiratory Flow, *ACT* Asthma Control Test

Exploratory outcome measures are all asthma-related

[a] Means and mean changes from V1 (baseline) will be compared between treatment groups at the specified timepoints

Table 3 Participant timeline

Procedures	Screening M0 ± 15 days	V1 Randomization—M1	V2 M2 + 15 days	V3 M3 + 15 days	V4 M4 + 15 days	V5 M5 + 15 days	V6 M6 + 15 days	V7 M7 + 15 days	V8 M8 + 15 days	V9 M9 + 15 days	V10 M10 + 15 days	V11 M11 + 15 days	V12 M12 + 15 days	V13 M13 + 15 days
Informed Consent Form	✓													
Baseline and Demographic Data	✓													
Medical History	✓													
Physical Examination	✓													✓
Haematology and Biochemistry	✓													✓
Serum Freezing for IgE and IgG analysis	✓							✓						✓
Instructions for medication usage	✓							✓			✓			
Participant Diary Assessment/ Revision	✓	✓						✓			✓			✓
Patient's Symptom and Medication Recording Assessment	✓	✓						✓			✓			✓
PEF assessment/ revision[a]		✓						✓			✓			✓
Recording of Exacerbations[a]		✓						✓			✓			✓
Pregnancy Test		✓												
Randomization		✓												
Mini-RQLQ, RCAT, ACT, VAS		✓						✓						✓
Exp. Drug/Pb. Administration		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
AEs Recording	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Direct and Indirect Costs Recording		✓						✓			✓			✓

M month, *V* visit, *PEF* Peak Expiratory Flow, *Mini-RQLQ* Mini Rhinitis Quality of Life Questionnaire, *RCAT* Rhinitis Control Assessment Test, *ACT* Asthma Control Test, *VAS* visual analog scale, *Exp.* experimental, *Pb.* placebo, *AE* adverse events

[a] Asthmatic patients only

revise the medical histories using the database of each hospital. Pre-selected patients will be invited to participate in the trial during the medical visit in the outpatient clinic. The investigators will, then, double-check that they meet all the inclusion and none of the exclusion criteria. If subjects accept to participate, they will be asked to sign the written informed consent before any study-related procedure is carried out.

Assignment of interventions: allocation

Sequence generation {16a}

The Sponsor will provide the trial randomization sequence. This will be computer-generated using random permutations based on pseudorandom number generators. Permutations will be done in blocks of size four. Treatment allocation will be done in a 1:1 proportion to either verum or placebo. The whole process will be stratified by presence/absence of asthmatic symptoms, so that the proportion of allocations is kept within each strata. All this will be done with the version 9.4 of the *SAS* software. Two copies of the randomization sequence will be produced. One will be kept in a sealed envelope that will be opened after the statistical analysis. The other will be sent to those responsible for packaging and labelling the investigational trial products. Concealed randomization lists (i.e. study groups will be identified as 'A' and 'B') will also be produced for use during the statistical analyses so that these can proceed without unblinding the treatments.

Concealment mechanism {16b}

The Sponsor will provide labelled trial products (all parcels and vials), ensuring complete concealment to all investigators and study participants. Vials will be visually identical, making it impossible to distinguish between the active product and placebo.

Implementation {16c}

Treatment parcels will be numbered. The principal investigator or a delegate investigator will randomize patients through an Interactive Web Response System (IWRS) and will receive a treatment parcel number corresponding to either the active product or placebo. This number will be displayed on the patient's individual prescription, which shall be taken to the pharmacy of the participating centres for medication dispensing.

Assignment of interventions: blinding

Who will be blinded {17a}

All participants and study team members will be blinded, including the PI, participating research physicians, personnel at the pharmacies of the study centres, statisticians, and outcome assessors.

Procedure for unblinding if needed {17b}

In case of a medical emergency or serious medical condition during a patient's participation in the trial, the PI or research physicians may perform an emergency unblinding for that patient. For this purpose, they will have access to an automated IWRS to find out the allocated treatments on an individual basis.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Figure 2 shows the main procedures to be carried out in each visit. The recording of AEs will be performed in each visit. Patients will be trained at baseline on how to use the phone application to complete a self-complete electronic diary including 4 nasal symptoms, 2 ocular symptoms and, for asthmatic patients, 3 asthma symptoms. The diaries will be also used to record the daily medication taken for allergy symptoms. They will be completed during the 4 weeks preceding visits V1, V7, V10, and V13, during which they will be checked for completion and integrity. Additionally, asthmatic patients will be trained on the use of the device to measure their PEF. Investigators will also check the correct use of the PEF metre. If mistakes are detected, they will take appropriate measures and retrain the patient if needed, and will try to correct the data and document the corrections in the eCRF during these visits. In addition, a Clinical Research Associate will perform weekly checks of the completeness of patients' diaries.

Patients finding it hard to complete the diaries, carry out other study-related tasks, or experiencing troublesome clinical situations may require extra visits. The investigators are allowed to perform such visits (either face-to-face or by phone).

Plans to promote participant retention and complete follow-up {18b}

The study duration for each patient will be of 14 months. They will be sent periodic reminders of the importance of complying with the study protocol through the study's self-complete electronic diaries. Furthermore, patients will receive reminders regarding the scheduled tasks, outstanding tasks, or planned study visits.

Data management {19}

The study team, overseen by each centre's PI, will be responsible for the accuracy, readability, detail level, and data collection deadlines. They will collect participants'

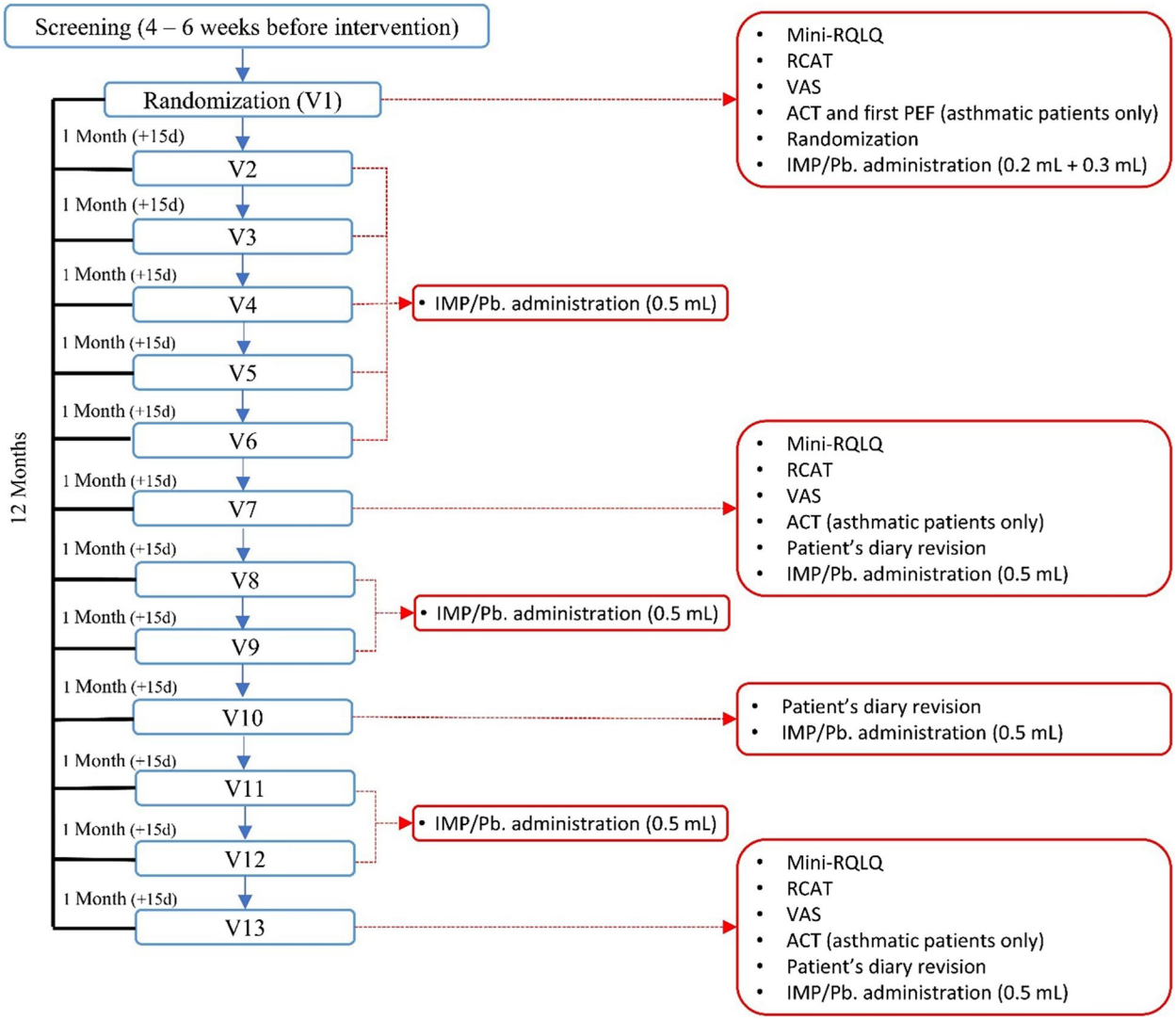

Fig. 2 Sequence of events. V: Visit; d: Days; PEF: Peak Expiratory Flow; Mini-RQLQ: Mini Rhinitis Quality of Life Questionnaire; RCAT: Rhinitis Control Assessment Test; ACT: Asthma Control Test; VAS: Visual Analog Scale; IMP.: Investigational Medicinal Product; Pb.: Placebo

source data; eCRF data must reflect the data gathered in the source documents.

Clinical and laboratory data will be collected on the eCRF, which will be monitored to identify errors in data collection. Each investigator will have a personal login and password to access the eCRF. Automated filters that include plausibility ranges will be deployed through the eCRF solution to ensure data consistency and integrity. Patients' self-assessed data will be collected through the validated phone applications developed ad hoc for the study. Each patient will have an individual login and password to access his/her personal space. The validated clinical tools to measure quality of life and control of rhinitis symptoms (mini-RQLQ and RCAT) will be administered on paper. The completed paper forms will be collected by the Clinical Research Associate in charge.

The data collected through the eCRF and the patients' applications will be stored in Windows Azure servers located within the European Union. The data recorded through paper questionnaires will be recorded in a validated *MS Access* database following double-entry robust procedures. In addition to automated filters, higher-order filters will be implemented using the *SAS* software. The data management team will issue Data Clarification Forms to the investigators on an ongoing basis to solve data issues. At study completion, a report will be prepared to list residual protocol deviations remaining after data filtering and cleaning, which will be used during the

database closure meeting to decide upon inclusion of patients in the analysis populations.

The Investigator and the Sponsor must store the collected data for at least 25 years after study completion.

Confidentiality {27}

All trial-related documents will be treated under the European Regulation (EU) 2016/679 of the European Parliament and Council (April 27, 2016) on Data Protection, as well as the Spanish Organic Law 3/2018 (December 5, 2018) on the Protection of Personal Data and Guarantee of Digital Rights. Patient data will be pseudonymized.

Each study participant will be assigned a unique study number to ensure anonymity, which will be used in the eCRF and the mobile phone applications. Regulatory Authorities, Trial Monitors, and Auditors may have direct access to study data, if required, and will take all possible precautions to maintain confidentiality.

The investigator is responsible for obtaining written informed consent from the study patients. The Trial Monitor will ensure that each patient has given written consent. The investigator shall ensure that the documents provided to the Sponsor do not contain the patient's name or any identifiable data.

All study participants will have the right to access, modify, oppose, and cancel data, as well as to limit incorrect data processing, request a copy, or transfer their data to a third party (portability). Patients will be provided with the study data protection delegate's contact (address, email, and phone number) along with the patient information sheet.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Biological samples obtained after the patient's informed consent will be stored in the Sponsor's sample collection for future research. These samples may be used to investigate the causes of IgE-mediated allergic disease, its complications, other conditions for which these individuals are at increased risk, and treatment improvements.

Participants are free to withdraw their consent for storing their samples for future research.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

All data will be described using the appropriate descriptive statistical methods, including means, SD, quartiles, minimum and maximum values for continuous variables, and absolute and relative frequencies for categorical variables. Additionally, 95% confidence intervals (95% CI) will be calculated to estimate the unknown population parameters.

Efficacy measures will be analysed using regression analyses based on the general linear or generalized linear model to accommodate the intra-patient structure of correlations intrinsic to repeated measurements over time. These models also allow adjustment for covariates.

We will use the general linear model whenever possible. Normality tests will be performed on the sample data by the Kolmogorov–Smirnov method, the Shapiro–Wilk method or both. If significant results are obtained, alternatives based on the generalized linear model shall be used.

The primary efficacy measure values at each visit will be obtained by averaging the daily values recorded in the participant diaries during the period corresponding to each visit. The primary outcome measure is the CSMS4, as recommended by the EACCI (see the Table 2 for a description). The CSMS4 shall be obtained repeatedly throughout the study. The main hypotheses (null and alternative) are defined as:

- H_0: $\{CSMS4_{Exp} - CSMS4_{Pb} \geq 0\}$
- H_1: $\{CSMS4_{Exp} - CSMS4_{Pb} < 0\}$

$CSMS4_{Exp}$ and $CSMS4_{Pb}$ refer to the scores of the aforementioned four-symptom rhinitis scale in the experimental and placebo groups, respectively, at any post-baseline study visit. Notably, this formulation implies a superiority test of the experimental treatment versus placebo. Such superiority may be tested for any post-baseline visit individually or for the entire scores' trajectory throughout the study.

The mixed models for repeated measures will include treatment, study visit, and site as fixed factors, baseline as a covariate, and patient as a random (intercept) factor. Other fixed factors may be incorporated that have prognostic value on the primary efficacy measure (which shall be specified in the SAP, if needed). We will use the estimate of the fixed treatment factor and the *p*-value associated with the Wald-type contrast for the hypothesis tests. The adjusted means for each treatment will also be provided together with their corresponding 95% CI. We will retain the interaction between treatment and visit to obtain the adjusted means per treatment and visit. If the centre factor is significant, we will also try to retain the interaction between treatment and centre (and present the means adjusted by centres) to assess possible differences in the effects between centres.

A logarithmic data transformation will be initially attempted if the normality assumptions are not met. If

normality assumptions remain unmet, we will use the generalized linear model with a Gamma distribution for errors and a (natural) logarithmic link functions. In such a case, the adjusted means would be retro-transformed to ease its interpretation.

Several secondary efficacy measures (CSMS, dSS4, dSS6, dMS) are similar to the primary efficacy measure. Hence, they will be analysed following the same procedure. The mini-RQLQ, RCAT, and VAS scores will also be analysed similarly to the primary efficacy measure. We do not expect the RCAT and VAS scores to deviate from normality and intend to use the general linear model; however, the VAS results may require a logarithmic transformation. If the normality remains unmet, we would proceed as described.

Disease-related costs will be calculated as the product of the number of times each healthcare unit resource cost was used by the corresponding resource's unitary cost (in euros, €); the accumulated costs will be compared at the end of the study. We will only analyse the data from patients who complete cost-related information in all follow-up visits and with at least 9 months of study participation (to avoid distortions caused by missing values due to dropouts), except in case of death. We will perform two unilateral *t*-tests to test for equivalence with the equivalence thresholds that will be specified in the statistical analysis plan (SAP). In addition, we will calculate a cost-effectiveness measure as the quotient between each patient's accumulated cost and the quality-adjusted life years (QALY) calculated from the mini-RQLQ. These measures represent the cost of obtaining a unit of effect (i.e. a QALY) with the corresponding treatment. Last, incremental cost-effectiveness ratios will be calculated with respect to the placebo control from the public insurance health perspective to ascertain whether and how the AIT under study affords benefits to the health system.

Safety measures will include the adverse events and laboratory data. Adverse events will be coded by Systems and Organs to the Lowest Level Term according to the Medical Dictionary for Regulatory Activities (MedDRA) and described by severity, seriousness, and relationship to investigational products. In addition, adverse reactions to the investigational products will be further classified as local or systemic according to the World Allergy Organization (WAO) grading system [21].

Safety data will be analysed on a safety analysis population comprising all randomized patients who receive at least one dose of the investigational product. Efficacy analyses will be performed over a modified intention-to-treat population that will be a subset of the safety population comprising patients who have both baseline and at least one post-baseline assessment of the primary outcome measure reported. If more than 10% of randomized patients had relevant protocol deviations, a per-protocol population would be defined to exclude these, and the efficacy analyses would be repeated in this subset to assess the influence of such deviations on the results.

The SAP will be made available and will further detail the statistical analyses. All statistical analyses will be performed using the version 9.4 or higher of the *SAS* software.

Interim analyses {21b}

No interim analyses are expected for this study with the exception of the blinded safety data summaries that will be provided at two prespecified timepoints to the Data Safety Monitoring Board (DSMB, see *item 21a*). No decision pertaining trial continuation or modification in any way will be taken as a result of the assessments by the DSMB.

Methods for additional analyses (e.g. subgroup analyses) {20b}

No subgroup analyses are initially planned. However, the possible influence of the patient's sex and age and the presence of asthma or other concomitant diseases on the results should be explored by introducing these variables into the regression models provided for the analysis of the efficacy measures. If significant interactions are detected in any of these models, we shall consider performing the corresponding subgroup analyses, but these will be always regarded as exploratory.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

We will perform sensitivity analyses if more than 20% of the participants have missing CSMS4 values on 20% or more of the days preceding each assessment visit. We will repeat the procedure described before excluding these patients.

Since the previous analyses concern models for repeated measures from each patient, these can accommodate random data missing mechanisms—i.e. not specifically related to the interventions, which constitute a reasonable assumption in this study. In the event of an aberrant pattern of missing values, for example, more than 20% of intermediate values erratically cancelled due to incorrect completion of the participant's diaries, we will apply imputation techniques using simple linear interpolation before running the analyses.

Suppose relevant between-participants differences are evidenced regarding their exposure times. In that case,

we may incorporate the visit factor into the aforementioned models as random instead of a fixed factor, which implies the incorporation of random slopes into the models. In this case, we would give further details in an update of the SAP.

As mentioned, efficacy analyses will be performed following the intention-to-treat ideal using an analysis set (herein called *modified intention to treat population*, see § 20a) that will include as much of randomized subjects as possible. The only exceptions, to safeguard the integrity of the analyses, will be patients who do not receive any dose of the IMP or do not have any data post randomization. This is considered to yield conservative estimates, since in general protocol non-adherence issues will tend to diminish the estimated treatment effect in a superiority trial like this.

Plans to give access to the full protocol, participant-leveldata and statistical code {31c}

The sponsor of the study will oversee the dataset and the data analysis. Granting access to this information will be allowed on a personalized basis, upon request by the interested party, and with permission from the corresponding author of this article, who will oversee the study. Data access requests should be addressed to the email address included in the article header.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

The study team will monitor the trial procedures, including participant recruitment, protocol adherence, and AEs/adverse reactions reporting.

The steering committee will oversee the project and meet at least once per month. It will decide the necessary actions to carry out the study according to the protocol and guarantee the interests of the study participants. The composition and responsibilities of the study team are described in the appropriate Sponsor's internal Standard Operating Procedures.

Composition of the data monitoring committee, its role and reporting structure {21a}

A DSMB will monitor and assess the safety of the trial and review the serious adverse events across the study duration. The DSMB will comprise of experts in clinical trials and allergy immunotherapy. All adverse events will be regularly reported to the DSMB members. A blinded interim safety analysis will be performed at 9 and 18 months following 15% ($n = 37$) and 60% ($n = 150$) patient recruitment, which results will be shared with the DSMB.

Adverse event reporting and harms {22}

AEs will be systematically assessed in all study visits. The investigators will actively inquire about any untoward medical event and will also collect events spontaneously reported by participants. These will be coded as described for the statistical analysis (see *Item 20a*).

Serious AEs (SAEs) will be reported by the investigator with an ad hoc SAE Notification Form and sent to the Sponsor by email within 24 h of event awareness. The Sponsor will notify the Spanish Medicines Agency (AEMPS) of any suspected unexpected serious adverse reaction (SUSAR) associated with the investigational product. This notification will be made through the EudraVigilance_CTM database of the European Medicines Agency (EMA).

The deadline for the Sponsor to report SUSARs to the AEMPS will depend on the reaction severity: (i) fatal or life-threatening SUSARs will be notified as soon as possible and, in any case, within 7 days after the Sponsor becomes aware of them; (ii) non-fatal and non-life-threatening SUSARs shall be notified no later than 15 days after the Sponsor becomes aware of them. Notably, fatal or life-threatening SUSARs initially not considered as such shall be notified as soon as possible and, in any case, within 7 days after the Sponsor becomes aware of their fatal or life-threatening nature. If needed, the Sponsor may make an initial incomplete SUSAR notification to ensure celerity; however, a complete report shall be made available no later than 8 days after the initial submission.

The sponsor will draft an annual Drug Safety Update Report (DSUR) that reassesses the safety of the IMP, taking into account all the available information. The DSUR will be made available to the AEMPS and IRBs and responsible health authorities of the relevant Spanish regions.

Frequency and plans for auditing trial conduct {23}

An independent Clinical Research Associate will perform regular monitoring visits to the study sites. If any major issue is detected during these visits, a full independent audit may be performed.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

As per good clinical practice, trial participants will be informed of any significant changes during the clinical trial. Major protocol amendments will be submitted by the sponsor to the IRB for approval and to the AEMPS; minor amendments will be notified to the IRB. Once approved, the amended protocol would be delivered to all participating sites and duly filed in the investigators'

study file. In any amendment is made to the materials for the informed consent, an updated consent would be obtained from all participants.

A trace will be kept if any major protocol deviation occurs by fully documenting it using dedicated breach report forms.

Dissemination plans {31a}

This clinical trial is registered in the EU Clinical Trials Platform, the Spanish Clinical Trials Registry (REec), and ClinicalTrials.gov. The results will be made public through these platforms within a maximum 1-year period after the end of the trial.

The results will also be sent for publication at allergy and clinical immunology conferences and in peer-reviewed scientific journals.

Discussion

This phase III clinical trial will be of interest to contribute to the scientific evidence about the efficacy and safety of AIT with allergoids. It has been designed according to both the EMA's [18] and EAACI [17] guidelines for the clinical development of products for the treatment of AR. This is not a minor issue given the aforementioned heterogeneity of the clinical research done to date for AITs. In 2008, the EMA stated that both symptom scores and medication scores should be assessed as primary endpoints, but left the question open as to whether they should be analysed separately or as a combined outcome. In this vein, the EAACI later on recommended using integrated and homogeneous CSMS as a straightforward and standardized method to balance both symptoms and the need for antiallergic medication with the aim to enhance comparability of results from different studies. We will use the CSMS4 as the primary outcome measure and, if there is sufficient statistical power, test its components (i.e. dSS and dMS) in a confirmatory way.

To obtain a complete picture of the effects of AIT, we will use additional disease control scores, quality of life scales, and serum biomarkers' concentrations to complete the secondary outcome measures. AEs will be systematically assessed for adverse reactions by the WAO grading system {Cox, 2010 #86} and by monitoring haematological and biochemical blood parameters.

We also expect to show that treating HDM-sensitized AR patients with Beltavac® for 12 months has a beneficial economic impact by reducing the direct and indirect costs of the disease. This analysis is of great interest for health insurance systems, especially because the data available so far in this respect for AITs is limited, as well as for the wider general framework of health technology assessment [24].

Notably, Beltavac® may also be beneficial to HDM-sensitized AR patients with associated allergic asthma. Thus, we created a combined scale based on the GEMA 5.0 guideline [13] to assess the severity of allergic asthma symptoms and medication requirements, analogous to the CSMS. Albeit exploratory, this assessment will provide interesting indicative data, because there is currently no validated tool to assess such symptoms and medication in allergic patients. This will be supplemented with more objective such as the number of exacerbations and the ACT questionnaire. Moreover, this will be the first phase III clinical trial of AIT that will monitor asthmatic patients' PEF daily thanks to the medical device and the phone application developed ad hoc.

The patients' self-complete electronic diaries will be fully integrated into the eCRF which will allow direct and real-time monitoring of compliance by patients, which we expect will improve adherence and data quality. Compliance and reliability have been common issues when patients were asked to fill in data daily for long periods of time. The application will automatically block the data 48 h after the date of filling. This will improve the integrity and enhance confidence of our data.

This study may have limitations. First, the CSMS is not yet clinically validated, despite its validation was warranted almost 10 years ago [17]. Notwithstanding, we plan also to perform some objective psychometric validation analyses using the data from this study, which we hope will inform of the measurement properties of the CSMS and be of great interest in the area of AITs for AR. Although the CSMS has a subjective component, we expect that the use of more objective outcomes, such as the proportion of symptom-free days or the validated tools for rhinitis symptoms (RCAT), will help in this task.

Trial status

Protocol version number: 4.0 (July 1st, 2022).
Recruitment Start date: January 17th,2022.
Recruitment End date: December 2025.
This trial is currently on the recruitment phase (August 28st, 2023).

Abbreviations

95% CI	95% Confidence intervals
AE	Adverse event
AEMPS	Spanish Medicines and Health Products Agency
AIT	Allergen immunotherapy
ARIA	Allergic Rhinitis and its Impact on Asthma
AR	Allergic rhinoconjunctivitis
ACT	Asthma Control Test
CRF	Case Report Form
CSMS4	Combined Symptom and Medication Score – 4 nasal symptoms
CSMS	Full Combined Symptom and Medication Score (4nasal + 2eye symptoms)
dMS	Daily Medication Score
DSMB	Data Safety Monitoring Board
dSS4	Daily Symptom Score – 4 nasal symptoms

dSS6	Full daily Symptom Score (4nasal + 2eye symptoms)
DF	*Dermatophagoides farinae*
DPT	*Dermatophagoides pteronyssinus*
DSUR	Drug Safety Update Report
eCRF	Electronic Case Report Form
EAACI	European Academy of Allergy and Clinical Immunology
EMA	European Medicines Agency
GEE	Generalized Estimating Equations
GEMA 5.0	Spanish Guideline for Asthma Management 5.0
GP	General Practitioner
HDM	House dust mites
IRB	Institutional Review Board
IMP	Investigational Medicinal Product
MedDRA	Medical Dictionary for Regulatory Activities
PEF	Peak Expiratory Flow
PI	Principal Investigator
QALY	Quality-adjusted life years
mini-RQLQ	Mini Rhinoconjunctivitis Quality of Life Questionnaire
RCAT	Rhinitis Control Assessment Test
SABA	Short-Acting Beta Agonists
SAEs	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SMD	Standardized mean difference
SUSAR	Suspected unexpected serious adverse reaction
VAS	Visual analog scale
WAO	World Allergy Organization

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Authors' contributions {31b}
The authorship is based on the criteria according to International Committee of Medical Journal Editors (ICMJE) (accessed on January 31 st, 2023).

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This study is funded by Probelte Pharma S.L.U. with the support of the Spanish Department of Science and Innovation (CDTI Programme).

Data availability {29}
The sponsor will keep the study database who, upon request by any interested party, may grant access to it. Data access requests should be addressed to the email address of the corresponding author included in the article header.

Declarations

Ethics approval and consent to participate {24}
The IRB of the Virgen de la Arrixaca Universitary hospital acted as the trial's coordinating ethics committee. The study protocol, version 1.1, was approved on September 7 th, 2021.

Consent for publication {32}
All authors have read and approved this manuscript.

Competing interests {28}
Inmaculada Buendia-Jimenez and Maria Matas-Ros are employees at Probelte Pharma. Teresa Garriga-Baraut, Albert Roger-Reig and Ana Tabar-Purroy have received honoraria for consulting in allergy research from Probelte Pharma.

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