Name of Sponsor/Company: Angany Inc.	
Name of Investigational Product: Fel d 1-eBP	
Name of Product: Fel d 1-eBP vaccine	
Active Ingredient: Fel-d-1 bioparticles colloidal solution which contains 12 ug/mL of a recombinant version of the major allergen of cat allergy Fel d 1 produced in the *Nicotiana benthamiana* plant system.	
Title of Study: An open label, safety and allergenicity Phase 0 study of a new hypoallergenic plant-derived cat dander vaccine in adult cat allergic subjects	
Study centre: Allergy and Clinical Immunology, Royal Brompton Hospital, London, UK	
Overall study period (months): 3 Estimated date first patient first visit: February 15, 2023 Estimated date last patient completed: May 15, 2023	**Phase of development:** Exploratory—Phase 0

Background and rationale:

Allergy to cats is a very common condition affecting 5–20% of the world population with almost all cat allergic subjects being sensitive to the major allergen Fel d 1. Immunotherapy by desensitization is the only disease-modifying treatment that offers the possibility of sustained efficacy; however, it only has a niche position because the treatment is long and intensive, and allergic side-effects are common and occasionally severe. Subcutaneous immunotherapy using crude standardized cat allergen extracts carry a risk of anaphylaxis, particularly in asthmatics.

The objective of immunotherapy is to direct the immune response away from a Th2-driven IgE-dominated humoral immune response towards a protective anti-inflammatory Th1/Treg-driven IgG1/IgG4-dominated one. Unfortunately, allergens are not antigens capable of rapidly inducing an effective and strong Treg/Th1-driven

protective blocking IgG response.

Angany has developed a plant-based manufacturing process to produce a high number of copies of allergens on bioparticles (eBioparticles™) which trigger a strong Th1/Treg-driven blocking IgG-immune response. These eBioparticles, including Fel d 1-eBPs, have been shown *in vitro* and *ex-vivo* to be both hypoallergenic and hyperimmunogenic and as such, a Fel d 1-eBP vaccine is a potentially safe and effective approach for immunotherapy for cat allergy. By reducing allergenicity and retaining/enhancing immunogenicity, the new Fel d 1-eBPs should act as a true vaccine, meeting an unmet need for a more effective and safer form of immunotherapy with fewer injections and a shorter course of treatment to induce long-term tolerance.

Overall study objectives:

The overall aim of this Phase 0 study is to determine the preliminary safety and allergenicity of the new Fel d 1-eBP vaccine in adult subjects with cat allergic rhinitis/rhinoconjunctivitis with or without mild to moderate (controlled) asthma. Study objectives will be met through the use of a standard diagnostic Skin Prick Test (SPT) as well as the Intradermal Skin Test (IDT).

Study objectives:

1. To assess the preliminary safety of the Fel d 1-eBP vaccine on skin prick and intradermal testing
2. To measure the degree of allergenicity of Fel d 1-eBP, in comparison to a Fel d 1—containing commercial cat dander extract diagnostic challenge test in a titrated SPT in adult cat allergic subjects
3. To evaluate the late phase immunological response at 6.5 hrs to intradermal injection of Fel d 1-eBP (IDT)

Primary study endpoint:

Safety: general safety of the new vaccine in skin testing will be assessed by evaluating adverse events (AEs), vital signs (body oral temperature, respiratory rate, heart rate, and blood pressure) and if needed, physical examination.

Secondary endpoints:

Skin reactivity/allergenicity: The early phase skin response (wheal diameter) to Fel d 1-eBP in a titrated SPT compared to a commercial cat dander allergen extract, will be evaluated. The primary outcome will be the provocation concentration of allergen that causes a ≥ 5 mm skin wheal. In addition to the early phase response, the late phase skin response following intradermal administration of Fel d 1-eBP will also be compared to the commercial extract at 6.5 hours post-injection.

The provocation concentration of allergen that causes a ≥ 3 mm skin wheal will also be determined, as a secondary endpoint.

Ex vivo **allergenicity assessments on blood samples:**

- Basophil Activation Test (BAT)—to determine the percentage of basophils activated in response to Fel d 1-eBP compared to a commercial cat dander extract.
- Diamine Oxidase (DAO) Test—to determine the potency of Fel d 1-eBP to induce basophil histamine release compared to a commercial cat dander extract.

Study Design and Procedures:

This Phase 0 study is an exploratory, open label, non-randomised SPT/IDT evaluation involving subjects with a history of a minimum of two years cat-induced allergic rhinitis/rhinoconjunctivitis with or without mild to moderate (controlled) allergic asthma.

At the screening visit (V0), all participants will have a medical history taken and a directed physical examination. FEV-1 will be measured first using appropriate spirometric equipment. Vital signs will be measured. SPTs (Soluprick HQ10, ALK Denmark) will be performed on the flexor aspect of one forearm with a panel of inhaled allergens including cat dander (in duplicate for eligibility assessment). Blood will be withdrawn for hematology, biochemistry, total IgE and allergen specific IgE (*ImmunoCAP* test) to whole cat allergen and major cat allergen Fel d 1. Blood samples will be obtained before SPTs are performed. Briefly, the following study procedures will be done in all subjects, as follows:

After an interval of a minimum of 7 days post screening (maximum 28 days), participants will return to the clinic in the morning (V1 visit). Use of prohibited meds will be verified. Vital signs will be measured. Extinction dilution SPTs will be performed in duplicate on the flexor aspect of both forearms (one arm for each product). Both the new experimental vaccine and commercial cat dander extract will be administered as successive dilutions of both products, ensuring equivalent concentrations of Fel d 1 allergen between the two products at each concentration. The respective Fel d 1 concentrations of the commercial extract (ALK Soluprick) and the experimental vaccine (Fel d 1-BP) are 41 and 12 µg/ml, respectively. Therefore, the following 6 dilutions of each product will be administered, in duplicate, ALK Soluprick: 1/3.4, 1/17, 1/170, 1/1,700, 1/17,000, 1/170,000; Fel d 1-BP: undiluted, 1/5, 1/50, 1/500, 1/5,000, 1/50,000. Two additional SPTs will be performed as well: 1 for the inert negative control, and 1 for a histamine positive control. This gives a total of 14 tests on each forearm, one used for for ALK Soluprick and controls, and the other one for Fel d 1-BP and controls.

Individual wheal sizes will be recorded and the mean of 2 measurements will be used. SPTs will be performed without any lag time in each participant.

Early and late-phase (T cell dependent) response will also be tested following intradermal tests (IDT). After recording the extinction dilution SPT results, IDTs will be performed on the extensor surface of each forearm. 1/1000 and 1/100 dilutions

of the provocation concentration of Fel d 1 allergen that caused a 3 mm skin wheal as judged by the previous same-day extinction dilution skin prick testing will be used for both Fel d1 eBP vaccine and commercial cat dander extract (on different arms).

If found to be sufficient and safe, then these same 2 doses will be given to the remaining participants.

The immediate test wheal size will be recorded at 15 minutes. Participants will remain in the allergy clinic, under clinical observation during 6.5 +/- 0.5 hours after the first IDT is performed, and will then undergo a late phase skin reaction evaluation, using a standard method. Patients will only be discharged after completion of all the safety assessments and if considered fit to leave the hospital.

A sentinel dosing strategy will be employed. A first group of three (3) subjects will complete all skin test procedures (SPTs and IDTs) as well as a 24-hour post-skin test safety follow-up. A minimum of 7 days will occur before initiating the skin tests in all remaining subjects. If the post IDT-skin reaction is not significant enough using the 1/1000 and 1/100 dilutions, then 1/100 an 1/30 dilutions will be used in 3 additional sentinel subjects, as well as in all remaining subjects.

In order to properly assess potential immediate and short-term adverse events, all patients will be closely monitored during 1 hour following all skin tests. Heart rate, blood pressure, body temperature, O_2 saturation and respiratory rate will be measured prior to SPT (at arrival) and 60 minutes after. Adverse events, if any, will be reported.

The Investigator will contact all subjects by phone approximately 24 hours following the end of the SPT/IDT session and again 7 days later to monitor for any adverse events and any changes in concomitant medications. Study participants will also be able to contact the study site any time after discharge, to report any safety question or issue.

Patient Population

Adults with a history of a minimum of two years of cat induced allergic rhinitis/rhinoconjunctivitis with or without mild to moderate asthma.

Sample size:

Twenty (20) evaluable cat allergic subjects (including the 3 sentinel subjects)

Diagnosis and main criteria for inclusion and exclusion (abbreviated list):

Inclusion criteria

Subjects must meet the following criteria to be entered into the study:

1. Adults (male or female) aged 18–60 years.
2. Documented recent (2 years) history of cat-induced:
    a. Moderate to severe persistent allergic rhinitis or rhinoconjunctivitis with or without:
    b. Allergic asthma (Global Initiative for Asthma (GINA ≤ Step 3)
3. A valid positive SPT (mean wheal diameter ≥7 mm obtained after screening, duplicate cat dander extract SPT) for cat.
4. Cat specific serum immunoglobulin E (IgE) measured by ImmunoCAP (≥1 kU_A/L).
5. Fel d 1 specific serum IgE measured by ImmunoCAP (≥1 kU_A/L).
6. Female subjects must be:
    a. of non-child-bearing potential [surgically sterilized or post–menopausal (12 months with no menses without alternative medical cause)] OR
    b. not pregnant, non breast feeding or planning to become pregnant AND willing to comply with the highly effective or effective contraceptive requirements of the study from Screening to at least 28 days after the last Investigational Medicinal Product (IMP) administration. Highly

effective and effective contraceptive methods include : combined hormonal contraceptives (pills, patch or vaginal ring), copper intrauterine device, tubal ligation, progestogen implant, levonorgestrel intra-uterine releasing system and depot medroxyprogesterone acetate SC or IM injections.

7. Able to speak, read and understand English sufficiently to understand the purposes and risks of the study and to provide written informed consent.
8. Willing, able and available to comply with all study procedures.

Exclusion criteria

Subjects who meet any of the following criteria will be excluded from the study:

1. History of current clinically significant gastrointestinal, hepatic, renal, cardiovascular, endocrine, oncological, immunological, neurological, ophthalmological, haematological, respiratory or psychiatric disorder or any other condition, which in the opinion of the investigator or sponsor would jeopardize the safety of the subject or the validity of the study results.
2. Severe or uncontrolled asthma as assessed by the GINA Asthma symptom control questionnaire OR current treatment for asthma at GINA> Step 3 OR screening FEV1 less than 80% predicted.
3. Subjects with a medical history of any previous episode of severe or life-threathening anaphylaxis or anaphylactic shock
4. Subjects with skin disorders that would hinder skin testing and/or its interpretation (e.g., severe generalized active atopic dermatitis).
5. Large tattoo(s) on the forearm, which could prevent the adequate assessment of wheal size, according to the investigator.
6. Any medical condition in which adrenaline (epinephrine) is contraindicated. A valid positive SPT (mean wheal diameter ≥7 mm obtained after screening,

duplicate cat dander extract SPT) for cat.

7. Female subjects must be:

    a. of non-child-bearing potential [surgically sterilized or post–menopausal (12 months with no menses without alternative medical cause)] OR

    b. not pregnant, non breast feeding or planning to become pregnant AND willing to comply with the highly effective or effective contraceptive requirements of the study from Screening to at least 28 days after the last Investigational Medicinal Product (IMP) administration. Highly effective and effective contraceptive methods include : combined hormonal contraceptives (pills, patch or vaginal ring), copper intrauterine device, tubal ligation, progestogen implant, levonorgestrel intra-uterine releasing system and depot medroxyprogesterone acetate SC or IM injections

8. Currently using or using within the specified timeframe of any of the list of prohibited drugs provided in this protocol.

Investigational medicinal product :

The Fel d 1-eBP vaccine will be supplied as a sterile suspension of 12 µg/mL Fel d 1 in PBS at pH 7.4

Reference product:

The ALK commercial cat dander extract (cat Soluprick 100,000 SQ) content of Fel d 1 of the lot to be used is 41 ug/ml. The lot to be used was assayed for Fel d 1 concentration prior to the study using an immunoblot test, in order to adjust the vaccine dilution factors. Sterile Sodium Chloride Injection BP 0.9% w/v will be used as well as the diluent.

Duration of Phase 0 :

9–29 days from screening to the end of safety follow-up, for each subject

Patient withdrawal and study stopping rules:

1. Individual patient stopping rules:

- Occurrence of at least 1 CTCAE grade 3 or 4 systemic adverse event of an at least *possible* causal relationship with at least one of the tested products 1 treatment emergent Serious Adverse Event (TESAE) of an at least *possible* causal relationship

2. Trial stopping rules

- Occurrence of a single Serious Adverse Reaction (SAR), regardless of type.
- Ocurrence of 2 Severe Adverse Drug Reactions (ADRs), regardless of type.

Statistical methods:

Sample size:

The study is exploratory as there is no preexisting data on which to base a power calculation. The study size (N=20) is based on a previous study where n=15-17 subjects was sufficient to detect a greater than 1 1⁄2 log (30-fold shift) to the right in the provocation concentration of allergen to cause a ≥ 5 mm skin wheal after subcutaneous grass pollen immunotherapy (*Durham et al., 1999*). Empirically a minimum of a 10-fold shift will be regarded as the minimal clinical important difference (MCID) and/or a reduction in mean plateau wheal diameter of ≥ 50%. comparisons of the plateau reaction among all 3 groups. The sizes of the intradermal skin early phase response (EPR) and LPR will also be compared according to the same hierarchy.

Sentinel dosing will be performed in 3 participants to assess the dose-response

and wheal sizes prior to performing Day 1 procedures in the remaining subjects.

A summary of the number and percentage of subjects with adverse events will be prepared, including adverse events by intensity, adverse events by relationship to Fel d 1-eBP, and serious adverse events. Adverse events will be coded using the MedDRA dictionary.

Vital signs (body temperature, respiratory rate, heart rate, oxygen saturation and blood pressure) will be summarized by timepoint. Changes over time in vital signs from pre-SPT will be summarized.

Physical examination data will be listed only.

The number and percentage of subjects receiving concomitant medications will be tabulated overall and by medication received.

Skin tests:

The primary skin reactivity/efficacy outcome will be the provocation concentration of allergen that caused a ≥ 5mm skin wheal. The results obtained for Fel d 1-eBP vs. commercial cat dander extract will be used for the primary analysis. Secondary analyses will include the provocation concentration of allergen that caused a ≥ 3 mm skin wheal, as well as comparisons of the plateau reaction among the 2 groups. The sizes of the intradermal skin early phase response (EPR) and LPR will also be compared according to the same hierarchy. The analysis will be an unpaired test using either T-test or Mann Whitney U-test depending on the distribution of the data being normal or skewed. A—2 degrees of freedom and a two-tailed test will be employed. There will be no correction for multiple comparisons.