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Study protocol of a phase 2, dual-centre, randomised, controlled trial evaluating the effectiveness of probiotic and egg oral immunotherapy at inducing desensitisation or sustained unresponsiveness (remission) in participants with egg allergy compared with placebo (Probiotic Egg Allergen Oral Immunotherapy for Treatment of Egg Allergy: PEAT study)

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ABSTRACT

Introduction Egg allergy is the most common food allergy in children but recent studies have shown persistence or delayed resolution into adolescence. As there is currently no effective long-term treatment, definitive treatments that improve quality of life and prevent fatalities for food allergies are required. We have previously shown that a novel treatment comprising a combination of the probiotic *Lactobacillus rhamnosus* CGMCC 1.3724 with peanut oral immunotherapy (OIT) is highly effective at inducing sustained unresponsiveness, with benefit persisting to 4 years after treatment cessation in the majority of initial treatment responders. In this study, we plan to extend the probiotic food OIT platform to another allergen, namely egg. We describe the protocol for a phase 2, dual-centre, randomised, controlled trial evaluating the effectiveness of probiotic and egg OIT at inducing desensitisation or sustained unresponsiveness (remission) in participants with egg allergy compared with placebo.

Methods and analysis 80 participants aged 5–30 years of age with current egg allergy confirmed by double-blind placebo-controlled food challenge at study screening will be recruited from Australia and Singapore. There are two intervention arms—probiotic and egg OIT (active) or placebo. Interventions are administered once daily for 18 months. The primary outcome is the proportion of participants who attain 8-week sustained unresponsiveness in the active group versus placebo group.

Strengths and limitations of this study

- ► This is the first double-blind placebo-controlled randomised trial to examine the effectiveness of probiotic and egg oral immunotherapy (OIT) in inducing desensitisation or sustained unresponsiveness in children with egg allergy compared with placebo.
- ► All participants will undergo a double-blind placebo-controlled food challenge at study entry to confirm diagnosis of egg allergy.
- ► Primary outcome of sustained unresponsiveness is assessed after 8 weeks egg elimination, which is longer than most published trials of egg OIT.
- ► Measurement of egg skin prick test, egg and egg component sIgE and $sIgG_4$ at study entry, end of treatment and 8 weeks post-treatment will provide information on immunological changes associated with probiotic and egg OIT and also with sustained unresponsiveness, desensitisation or persistent allergy.
- ► Comparison with egg OIT cannot be performed as this study has no egg OIT alone arm.

Ethics and dissemination This study has been approved by the Human Research Ethics Committees at the Royal Children's Hospital (HREC 2019.082) and the National Healthcare Group Domain Specific Review Board (2019/00029). Results will be published in peer-reviewed journals and disseminated via presentations at international conferences.

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INTRODUCTION

Background

Food allergy is a major public health problem in western countries,[1 2] affecting 8% of children[3] and 10% of infants.[4] There is no cure, so management relies on food avoidance. Despite mandatory food-labelling laws, accidental ingestion is common, causing frequent and sometimes life-threating or fatal reactions.[5 6] As allergic reactions occur as a result of unintentional ingestion and are therefore unpredictable, there is significant psychological distress and impact on quality of life (QoL).[7] A curative treatment offers the only approach to improve the lives of people with food allergy and to avoid deaths. Egg allergy is of greatest concern because it is the most common food allergy in childhood[8–10] affecting up to 8.9% of infants in Australia,[4] and 0.5% –2.5% of children worldwide.[3 8–10] Previous understanding of the natural resolution of egg allergy was that a majority of children would develop tolerance by school age. However, in recent studies, the rate of resolution of egg allergy appears to be delayed, with 42% of cases persisting into adolescence (>12 years old).[11]

The current management of egg allergy involves avoidance of the food concerned, early recognition of symptoms of an allergic reaction and initiation of appropriate emergency treatment of allergic reactions, particularly anaphylaxis. However, egg is a common ingredient used for cooking worldwide, as well as in a wide range of manufactured products, making dietary avoidance a challenge for families and a great risk to the patients.[12] In addition to current management, recent international guidelines and national expert societies (Spain, Canada) have suggested that oral immunotherapy (OIT) be considered for persistent egg allergic patients.[13–15]

There are two beneficial outcomes that can be achieved by a food allergy treatment: (1) desensitisation and (2) sustained unresponsiveness.[16 17] Desensitisation is the temporary increase in reaction threshold that is only maintained with regular ongoing treatment (antigen exposure). Sustained unresponsiveness, in contrast, refers to a long-lasting ability to tolerate standard serves of a food even after a period of treatment withdrawal; this is thought to reflect reprogramming of the immune response to allergen. Tolerance is the permanent resolution of allergy, essentially a cure and remains the optimal goal of treatment; however, permanence cannot be demonstrated in the setting of a clinical trial. Desensitisation may not be an optimal outcome of patients because individuals who are desensitised (without sustained unresponsiveness) can experience allergic reactions while continuing on treatment and reactions occur more frequently than with food avoidance.[18–21] Nevertheless, two treatments for peanut allergy that induce desensitisation without tolerance are being developed commercially and have completed phase III trials (DBV Viaskin patch; Aimmune AR101 OIT[22 23] where one has received Food and Drug Administration (FDA) approval.[24] In peanut OIT, sustained unresponsiveness has been shown in a high proportion of younger children aged 1–3 years.[25] For food immunotherapy trials, desensitisation can be assessed by performing a double-blind, placebo-controlled food challenge (DBPCFC) while the subject is on treatment, whereas sustained unresponsiveness can be assessed by DBPCFC performed several weeks after treatment has been withdrawn.[16 17] National Institute of Allergy and Infectious Diseases (NIAID)-FDA guidelines for food allergy clinical trial design recommended that when assessing for sustained unresponsiveness in food immunotherapy trials, DBPCFC should be performed at least 2–4 weeks after treatment is ceased.[26] However, it was reported that some subjects who achieved sustained unresponsiveness at 1 week after withdrawal of treatment subsequently lost this protection by 6 weeks.[27] Newer studies have, therefore, elected to wait a longer period of time (at least 4–8 weeks after treatment withdrawal) before performing DBPCFC to demonstrate sustained unresponsiveness with greater confidence.[28 29]

Studies evaluating egg OIT have reported desensitisation in 57%–94% of treated participants, however, the ability to induce sustained unresponsiveness is limited (<30%).[30] The first randomised trial of egg OIT in 55 children aged 5–11 years old (n=40 egg OIT n=15 placebo) reported desensitisation in 55% of OIT treated children compared with 0% placebo, and sustained unresponsiveness in 28% of OIT treated children and 0% of placebo after 22 months of treatment.[28] A subsequent randomised trial conducted in 31 children aged 4 to 11 years (n=17 egg OIT, n=14 placebo) showed sustained unresponsiveness in 31% of children who received egg OIT compared with 7% of placebo-treated children.[29]

A new class of tolerogenic compounds that modulate immune responses by acting on antigen-presenting cells through Toll-like receptors, so called immune response modifiers (IRM), have been used with allergen immunotherapy to promote tolerance responses in allergic airway disease.[31–33] The probiotic *Lactobacillus rhamnosus* American Type Culture Collection (ATCC) 53103 is an IRM with immunomodulatory effects in vitro and in vivo that are associated with oral tolerance, including induction of Treg and Th1 cytokine responses[34–37] and enhanced antigen-specific IgA responses[38 39]; however it remains unknown whether these immune changes can support the acquistion of tolerance in allergic patients. We therefore postulated that coadministration of *L. rhamnosus* ATCC 53103 with a food antigen would be effective for induction of oral tolerance or sustained unresponsiveness to that food. This premise is supported by our previous finding that combined administration of probiotic *L. rhamnosus* ATCC 53103 with peanut antigen was effective at induction of sustained unresponsiveness to peanut after 18 months of treatment.

The Probiotic and Peanut Oral Immunotherapy (PPOIT) study (PPOIT-001) evaluating PPOIT in

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children with peanut allergy was a double blind placebo controlled randomised trial, which resulted in the best response rates yet reported for any food allergy therapy in development.[40] Sixty-two children with peanut allergy were randomised to receive PPOIT or placebo for 18 months; 82% of PPOIT treated participants achieved sustained unresponsiveness (remission of peanut allergy) as compared with only 3.6% of placebo-treated children. Furthermore, clinical benefit was long-lasting; 80% of PPOIT-treated children who achieved sustained unresponsiveness at the end of treatment were still tolerating peanut 4 years after end of treatment.[41] Our data indicate that PPOIT can induce long-lasting sustained unresponsiveness to peanut in children with peanut allergy, is safe, and effects are allergen specific. A limitation of the PPOIT-001 study was the lack of an OIT only arm. Further work to clarify the contribution of probiotic over OIT alone is currently underway.

We now wish to investigate whether this combination probiotic food OIT approach is effective for the treatment of other life-long food allergies such as egg allergy. If probiotic egg OIT is shown to be effective at inducing sustained unresponsiveness, we will have established that probiotic food OIT offers a platform approach for treatment of other food allergies. This study will produce the proof of concept needed to advance further phase 2 studies of probiotic food OIT for the induction of sustained unresponsiveness in egg and other food allergies.

This paper reports the research protocol for a phase 2, dual-centre, randomised, controlled trial evaluating the effectiveness of probiotic and egg OIT at inducing desensitisation or sustained unresponsiveness (remission) in participants with egg allergy compared with placebo.

Objectives

Primary objective

1. To compare the proportion of subjects who attain 8-week sustained unresponsiveness (passed T1 and T2 challenges) in active and placebo groups.

Secondary objectives

1. To compare the proportion of subjects who achieve full desensitisation at end of treatment (passed T1 challenge) in active and placebo groups.
2. To compare the total cumulative dose of egg white protein tolerated during the end of treatment T1 challenge in active versus placebo.
3. To compare change from baseline in egg skin prick test (SPT) weal size at the end of treatment, and 8 weeks after end of treatment in active and placebo groups.
4. To compare change from baseline in sIgE and $sIgG_4$ levels to egg and egg components (Gal d 1, 2, 3) at end of treatment, and 8 weeks after end of treatment in active and placebo groups.
5. To compare change from baseline in QoL at end of treatment, and 8 weeks after end of treatment—measured using validated QoL questionnaires in active and placebo groups.
6. To evaluate the safety and tolerability of probiotic and egg OIT.

METHODS AND ANALYSIS

Study design

This is a phase 2, dual-centre randomised (1:1), controlled trial evaluating the effectiveness of probiotic and egg OIT at inducing desensitisation or sustained unresponsiveness (remission) in children and adults with egg allergy compared with placebo (Probiotic Egg Allergen Oral Immunotherapy for Treatment of Egg Allergy: PEAT study).

Group 1. Active=Probiotic and egg OIT taken daily for 18 months.

Group 2. Placebo=Probiotic placebo and OIT placebo taken daily for 18 months.

Study setting

The is a dual centre study conducted at two sites—the Royal Children's Hospital Melbourne (RCH)/Murdoch Children's Research Institute (MCRI) in Australia and the National University Hospital (NUH), Singapore. RCH/MCRI and NUH will enrol 40 participants at each site (total sample n=80). Participants will be recruited from Allergy departments at these tertiary hospitals, from the general community via media outreach, and direct contact with community and relevant special interest groups as well as paid advertising. On expressing initial interest in joining the study, the participant and/or their parent/guardian will be contacted to assess suitability for the study.

DBPCFC, initiation and updosing of immunotherapy will be performed in hospital/clinical research facility by nursing and medical staff experienced in the performance of food challenges, immunotherapy, and management of allergic reactions.

Interim doses of immunotherapy will be administered at home. All subjects will be provided with an Anaphylaxis Action Plan, an Epinephrine autoinjector and educated in the management of allergic reactions (standard care for egg allergy). If a reaction occurs, they will follow the Anaphylaxis Action Plan, and notify the On-Call Study Personnel at the local study site.

Participants and eligibility criteria

Eighty participants between 5 and 30 years of age with current egg allergy confirmed by failed DBPCFC at study screening. Participants will be randomised to active (n=40) or placebo (n=40).

Inclusion criteria

Subjects are eligible for the study if they meet all of the following criteria:

- Aged between 5 and 30 years of age.
- Confirmed diagnosis of egg allergy as defined by a failed DBPCFC to egg.

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- A positive SPT or sIgE to egg at screening (A positive SPT is defined as weal size ≥3 mm and a positive sIgE is defined as ≥0.35 kUA/L).

Exclusion criteria

Subjects are not eligible for the study if they meet any of the following criteria:

- History of severe anaphylaxis (as defined by persistent hypotension, collapse, loss of consciousness, persistent hypoxia or ever needing more than three (3) doses of intramuscular epinephrine or an intravenous epinephrine infusion for management of an allergic reaction).
- Severe anaphylaxis during the study entry DBPCFC (defined as persistent hypotension, collapse, loss of consciousness, persistent hypoxia or requiring more than three doses of intramuscular epinephrine or an intravenous epinephrine infusion for management of an allergic reaction).
- Forced expiratory volume in 1 second (FEV1) <85% predicted at rest and FEV1/forced vital capacity ≤85% predicted at rest (for those participants able to perform spirometry testing) or current chronic persistent asthma (as per Australian Asthma Council guidelines).
- Underlying medical conditions (eg, cardiac disease) that increase the risks associated with anaphylaxis.
- Use of beta-blockers and ACE inhibitors.
- Inflammatory intestinal conditions, indwelling catheters, gastrostomies, immunecompromised states, post-cardiac and/or gastrointestinal tract surgery, critically ill and those requiring prolonged hospitalisation or other conditions that may increase the risks of probiotic associated sepsis.
- Already taking probiotic supplements or food containing probiotics in the last month.
- Reacting to the placebo component during the study entry DBPCFC.
- Have received other food immunotherapy treatment in the preceding 12 months.
- Currently taking immunomodulatory therapy (including allergen immunotherapy).
- Past or current major illness that in the opinion of the Site Investigator may affect the subject's ability to participate in the study for example, increased risk to the participant.
- History of suspected or biopsy-confirmed eosinophilic oesophagitis.
- Subjects who in the opinion of the Site Investigator are unable to follow the protocol.
- Another family member already enrolled in the trial (to maintain safety and blinding).
- Non-English speaking participants and families.
- Subjects who are on an egg ladder diet (except baked egg).

Patient recruitment, study procedure and data collection

The screening DBPCFC for the trial started in October 2019 and the planned end date is April 2023.

Consent procedure

Participants and/or their parent/guardian who are identified as potentially suitable to participate in the study will be sent a soft copy of the human research ethics board and institution-approved information statement and consent form.

Prior to full study enrolment or performing any study specific procedures (eg, screening DBPCFC), a signed consent form will be obtained from the participants if over 18 years old or from the parent(s) if under 18 years of age (cut-off is 21 years of age in Singapore). For the participants over 12 years old, the opportunity to sign a participant information and consent form will also be offered.

Randomisation and concealment mechanism

Participants will be enrolled and randomised up to 1 week prior to Rush Induction, and within 8 weeks of their Screening appointment. Randomisation will be to active or placebo groups with an allocation ratio of 1:1. Randomisation will be stratified by study site (RCH or NUH) and by SPT (≥10 mm and <10 mm). Randomisation will be in randomly permuted blocks of variable length. An independent statistician in the Clinical Epidemiology and Biostatistics Unit (CEBU) at the MCRI will provide the randomisation schedules to hospital pharmacies (or appropriate delegates) at each site. If a participant fails screening and is not randomised, or discontinues from the trial after randomisation, that participant's screening number and/or randomisation number will not be reallocated.

Participant eligibility will be established prior to enrolment and randomisation. A unique participant screening number will be allocated to each consented participant prior to proceeding with study screening. Participants who are confirmed as eligible for the study after the screening visit (including having failed the screening DBPCFC) will have an appointment made for Rush Induction and study personnel will notify the pharmacist that the participant is eligible for enrolment and randomisation.

Participants will be enrolled into the trial in strict sequence as their eligibility for enrolment is determined. Randomisation and enrolment will only be performed up to 1 week prior to Rush Induction and only randomised participants will commence Rush.

The pharmacist (or appropriate delegate) will assign the next available unique randomisation number for the participant's appropriate stratum using the randomisation list and notify the trial personnel of that number. This randomisation number will be recorded in the participant's source data. The pharmacist will prepare the participant's allocated study treatment and label the treatment with the participant's randomisation number.

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Participants, outcome assessors, other research staff, treating clinicians, investigators and trial statistician will be blinded to treatment allocation.

Probiotic (or placebo) and egg (or placebo) OIT regimen

Rush induction visit (T0): day 1

In this visit, participants will receive a single dose of 2×10^{10} cfu *L. rhamnosus* ATCC 53103 or placebo, followed by four increasing doses of egg or placebo OIT until the top tolerated dose is reached.

Egg OIT will be egg white protein powder that is prepared by Good Manufacturing Practice certified facilities. Placebo OIT will be maltodextrin.

Participants who complete the Rush protocol without reaction will commence the buildup phase at a daily dose on the day after the Rush Induction day. However, if a participant reacts to one of the doses during Rush Induction, the Rush schedule will be ceased and they will commence the Buildup Phase at the dose immediately below the reaction-eliciting dose starting on the day after the Rush Induction day.

Buildup phase

In the buildup phase, the daily dose of egg OIT (or placebo OIT) will be increased every 2 weeks until a maintenance dose of 1870 mg egg white protein is reached. Each dose increase will be administered in hospital under medical supervision.

Participants will also take a fixed dose of 2×10^{10} cfu *L. rhamnosus* ATCC 53103 or placebo once daily prior to the OIT treatment.

Parents will maintain a daily diary record of dosing, compliance, reactions to study product and any treatments administered for reactions during the whole time of the study.

Maintenance phase

In this phase, participants will take a daily dose of 1870 mg egg white protein and a daily dose of 2×10^{10} cfu *L. rhamnosus* ATCC 53103 or placebo until a total of 18 months of treatment is completed.

Clinical outcome

Sustained unresponsiveness at time point T2

Sustained unresponsiveness will be assessed by DBPCFC performed at 8 weeks after cessation of study treatment. The procedure of DBPCFC will be the same as described in the screening visit. Only those participants who pass the DBPCFC at the T1 visit will proceed to a DBPCFC during the T2 visit. Sustained unresponsiveness is defined as passing both the T1 and T2 DBPCFCs.

Full desensitisation at time point T1

Desensitisation will be assessed by DBPCFC performed 1 day after the last day of treatment (time point T1). Subjects who pass the T1 DBPCFC will be considered to have achieved full desensitisation.

Study outcomes

Primary outcome

- Proportion of participants who attain 8-week sustained unresponsiveness (remission) (passed T1 and T2 challenges) in active and placebo treated groups.

Secondary outcomes

- Proportion of participants who attain full desensitisation at end of treatment (passed T1 challenge) in active and placebo groups.
- The cumulative dose tolerated during T1 challenge (cumulative doses below the reaction-eliciting dose if there is a reaction; or total cumulative challenge dose if there is no reaction) in active versus placebo.
- Change from baseline in egg SPT at end of treatment, and 8 weeks after end of treatment in active and placebo groups.
- Change from baseline in serum/plasma levels of sIgE and $sIgG_4$ to egg and egg components (Gal d 1, 2, 3) at end of treatment, and 8 weeks after end of treatment in active and placebo groups.
- Change from baseline in QoL at end of treatment, and 8 weeks after end of treatment in active and placebo groups.
- Safety and tolerability of probiotic and egg OIT.

Study outline is described in figure 1.

Study visits

Screening visits

The following assessments will be conducted at the screening visit:

- Written informed consent.
- Confirm inclusion/exclusion criteria.
- Medical history and physical examination.
- Allergy questionnaire.
- Vital signs (blood pressure, pulse, respirations, temperature).
- Spirometry or peak flow (all participants 8 years and older).
- Weight/height.
- QoL questionnaires.
- SPT (egg, milk, peanut, house dust mite, rye grass, cat and positive and negative control).
- Blood sample.
- DBPCFC.

Rush induction: T0 visit

The following assessments will be conducted at the day 1 Rush Induction visit (T0):

- Allergy questionnaire.
- Vital signs (blood pressure, pulse, respirations, temperature).
- Spirometry or peak flow (all participants 8 years and older).
- Weight/height.
- Faecal sample (collected at home).
- Anaphylaxis education.
- Dispense subject diary.

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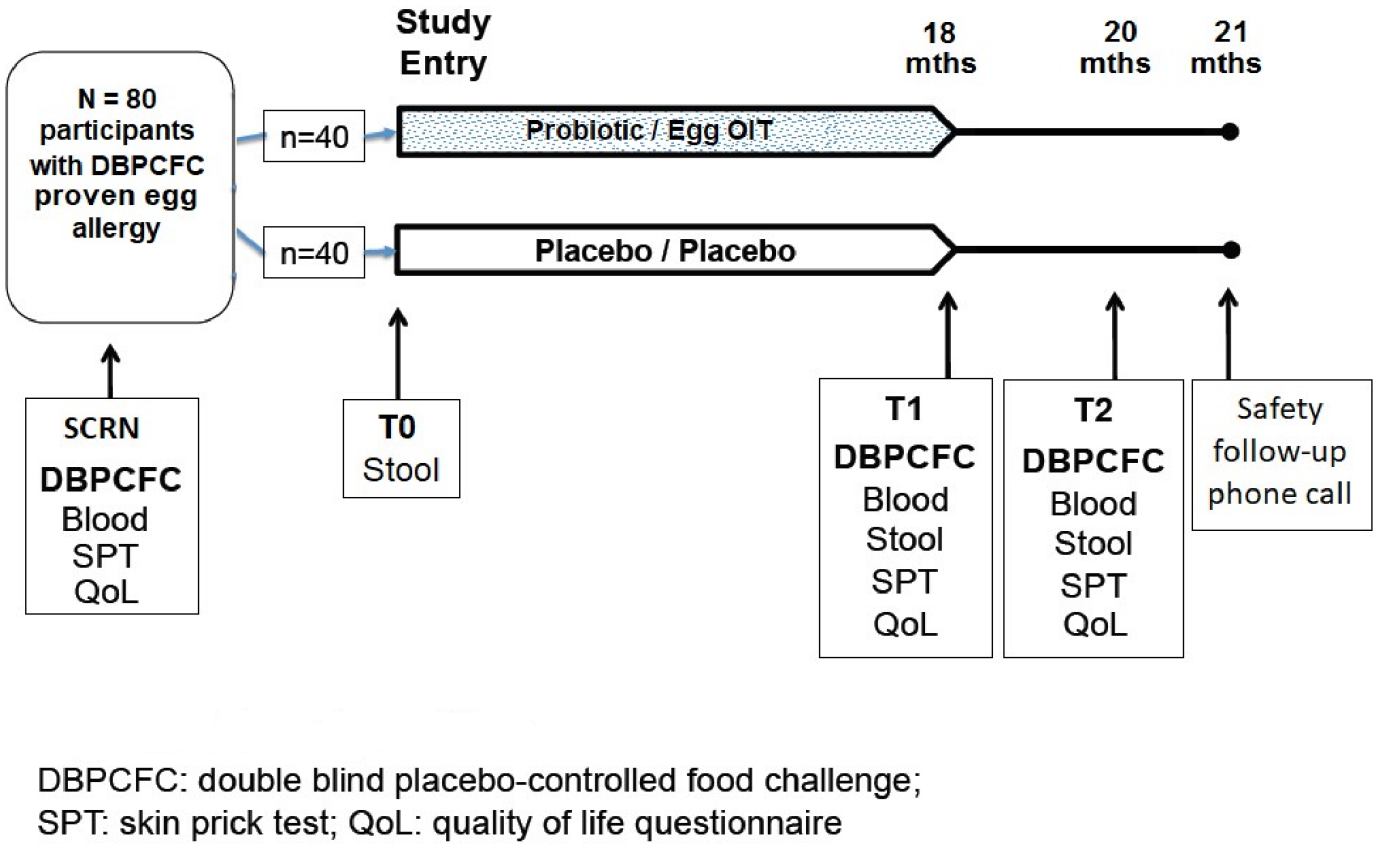

Figure 1 Study outline of the PEAT Study. OIT, oral immunotherapy; PEAT, Probiotic Egg Allergen Oral Immunotherapy for Treatment of Egg Allergy.

- Anaphylaxis action plan and epinephrine autoinjector (eg, EpiPen) provided.
- Dispense OIT/placebo and probiotic/placebo.

Buildup phase: visits

The following assessments will be conducted at these visits:

- Review adverse events and concomitant medications.
- Allergy questionnaire.
- Vital signs (blood pressure, pulse, respirations, temperature).
- Weight/height.
- Anaphylaxis education.
- Review subject diary.
- Collect study treatments for review of compliance.
- Dispense OIT/placebo and probiotic/placebo.

Maintenance phase: visits (every 3 months)

The following assessments will be conducted at these visits:

- Review adverse events and concomitant medications.
- Allergy questionnaire.
- Vital signs (blood pressure, pulse, respirations, temperature).
- Weight/height.
- Anaphylaxis education.
- Review subject diary.
- Provide faecal collection tube and instructions (for collection at T1).
- Collect study treatments for review of compliance.
- Dispense OIT/Placebo and probiotic/placebo.

End of treatment: T1 visit

There will be a study visit at 18 months (T1, end of treatment) for assessment of desensitisation.

The following assessments will be conducted at this visit:

- Medical review and physical examination.
- Allergy questionnaire.
- Vital signs (blood pressure, pulse, respirations, temperature).
- Spirometry or peak flow (all participants 8 years and older).
- Weight/height.
- QoL questionnaires.
- SPT (egg, milk, peanut, house dust mite, rye grass, cat and positive and negative control).
- Anaphylaxis education.
- Review subject diary.
- Collect study treatments for review of compliance.
- Review adverse events and concomitant medications.
- DBPCFC.
- Blood and faecal sample (faecal sample collected at home).

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- Provide faecal collection tube and instructions (for collection at T2).

Eight weeks after end of treatment: T2 visit

There will be a visit at 8 weeks after T1 for assessment of sustained unresponsiveness.

The following assessments will be conducted at this visit:

- Review adverse events and concomitant medications.
- Medical review and physical examination.
- Allergy questionnaire.
- QoL questionnaires
- Vital signs (blood pressure, pulse, respirations, temperature).
- Spirometry or peak flow (all participants 8 years and older)
- Weight/height.
- SPT (egg, milk, peanut, house dust mite, rye grass, cat and positive and negative control).
- Review & collect subject diary.
- DBPCFC if required.
- Blood and faecal sample (faecal sample collected at home).
- Anaphylaxis education.

Thirty days safety follow-up phone call after T2 visit

The following assessments will be conducted at this safety follow-up phone call:

- Review adverse events and concomitant medications.
- Allergy questionnaire.

Study procedures

Double blind placebo-controlled food challenge

Each DBPCFC will comprise two parts performed on two separate days, which are completed within 1 week of each other. The cumulative amount of egg white protein or placebo powder administered during the DBPCFC is 3982.3 mg (~1 large egg white) (table 1).

The doses will be administered at 15 min intervals, if the subject has not had a reaction consistent with a predefined stopping criteria (box 1) to the previous dose. The subject will be observed for a minimum of 2 hours following the DBPCFC and will be discharged home if no adverse reactions are noted.

Table 1 Food challenge doses

Dose	Dose egg white protein/placebo (mg)	Cumulative dose egg white protein/placebo (mg)
1	29.8	29.8
2	59.5	89.30
3	119.0	208.30
4	238.0	446.30
5	476.0	922.30
6	1020.0	1942.30
7	2040.0	3982.30

Box 1 Cessation criteria for DBPCFC

Any of the following objective signs occurring within 2 hours of ingestion:

- Three or more concurrent non-contact urticaria persisting for at least 5 min.
- Perioral, periorbital or facial angioedema.
- Vomiting (excluding gag reflex) and/or diarrhoea.
- Wheeze (either audible (without stethoscope) or on auscultation with stethoscope), change in voice, stridor, difficulty breathing.
- Persistent cough (ie, not just intermittent and transient throat clearing).
- Long bursts of sneezing/persistent rhinorrhoea (persistent defined as on three or more doses or more than 40 min).
- Collapse, hypotension.
- Prolonged severe abdominal pain* for 40 min.
- Severe generalised marked erythema (>50%).
- Three or more subjective symptoms requiring a dose delay criteria (ie, persistent throat tightness/pain (subjective), severe abdominal pain (subjective) and/or notably distressed due to gastrointestinal symptoms with decreased activity, mild subjective cardiovascular response (weak, dizzy) without evidence of hypotension or tachycardia, <3 non-contact urticaria or hard continuous scratching leading to excoriations, intermittent bursts of sneezing (<10), frequent sniffing, moderate areas of erythema (<50%)).

*Severe abdominal pain defined as >6 on Wong-Baker FACES scale or a physician assessment of severity for younger children.
DBPCFC, double-blind placebo-controlled food challenge.

Food challenge protocol and stopping criteria are shown in table 1 and box 1.

The DBPCFC will be classified as:

- 'Failed' if there is a reaction to the egg white component and no reaction to the placebo component (pharmacy will only un-blind the contents of part A and part B after both parts A and B are completed and provided the participant has failed one part and not the other part of the DBPCFC).
- 'Passed' if both parts A and B of the challenge are completed without reaction. The contents of parts A and B will not be un-blinded.
- 'Inconclusive' if participant reacts to both parts A and B (contents of part A and part B will not be unblinded) or if participant reacts to the placebo component but not the active component.

Severity grading for allergic reactions is based on the National Institutes of Health (NIH) NIAID Consortium for Food Allergy Research specific grading system for allergic reactions.

SPT and laboratory tests

At the time of the screening visit, as well as the end of treatment (T1), and at 8 weeks (T2) after end of treatment, up to 50 mL of blood will be collected for the measurement of specific IgE (sIgE) and specific IgG4 ($sIgG_4$) against egg and egg components (Gal d 1, 2, 3) by ImmunoCAP (Phadia AB, Uppsala, Sweden). Plasma and peripheral blood mononuclear cells will be isolated

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and stored at −80°C or in liquid nitrogen, respectively, for exploratory immunological studies.

SPT for egg, milk, peanut, house dust mite, rye grass (perennial), cat and positive and negative control will be performed at the same times as blood collection. Stool samples will also be collected at various times and stored at −80°C for future microbial studies.

Participant compliance

Participants will be asked to bring their study medication to each study visit. Compliance will be monitored by diary record of dosing as well as by treatment capsule/tub counts.

Adverse events reporting

Adverse events will be recorded from signed consent until the 30-day follow-up phone call after the T2 visit. Participants will be able to record any concern or adverse event in the diary for review at each study visit. Causality will be assessed by study doctors, using the following categories: unrelated, unlikely to be related, possibly related and probably related. The severity of an adverse event will be assessed and categorised according to whether the event is an allergic reaction or a non-allergic reaction. If the adverse event is an allergic reaction, the severity of the event will be categorised based on criteria adapted from the NIAID Consortium for Food Allergy Research specific grading system for allergic reactions. For all other adverse events (ie, events which are not allergic reactions), the severity of the event will be classified according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.

Statistical methods

Sample size and power calculation

The study sample size will be 80 participants, randomly allocated in a 1:1 ratio to active (n=40) and placebo (n=40).

Allowing for a 15% drop-out rate, 40 participants in each group provides 90% power to detect the difference between a 28% rate of sustained unresponsiveness in the placebo group (expected rate of natural resolution of egg allergy over a 2-year period in children aged 5–17 years) and a 70% rate in the active group, using a two group chi2 test, applying the continuity correction to the normal approximation of the discrete distribution, with 0.05 two-sided significance.

Statistical analysis

Data handling, verification and analysis will be performed within the CEBU at MCRI, and by representatives contracted by the Sponsor. Statistical analysis will follow standard methods for randomised trials and the primary analysis will be by intention to treat. All demographic and baseline continuous outcomes will be presented as mean and SD, median and IQR if not normally distributed, while categorical outcomes will be presented as absolute and relative frequencies in the two groups. For dichotomous outcomes (primary aim and secondary aims 1), between group comparisons will be presented as the risk difference and 95% CI at each time point, obtained using a binomial regression model, with adjustment for the centre, age at baseline and egg SPT weal size. Continuous outcomes (secondary aims 2–6) will be compared between groups using differences between mean values, estimated from normal linear regression models with the same adjustments outlined above. Egg SPT weal size, as well as egg and egg component sIgE and sIgG4 levels will be reported as mean and SD by treatment group. If continuous outcomes do not follow normal distributions they will be summarised as median and IQR in the two groups, and comparison between groups will be performed by the Wilcoxon rank-sum (Mann-Whitney U) test.

Primary outcome

The primary outcome assessment is whether a participant has acquired sustained unresponsiveness (passed T2 challenge). Results will be summarised as the number and proportion of participants with 8-week sustained unresponsiveness (remission) in the two treatment groups. Comparison between active with placebo will be presented as the absolute and relative risks, accompanied by the respective 95% CI obtained using a binomial regression model, with adjustment for the centre, age at baseline and egg SPT weal size.

Subgroup analysis

As a secondary analysis on the primary and secondary outcomes, we will examine the effect of the interactions between treatment received and the following: site (Melbourne vs Singapore) and age (5–15 vs 16–30 years old at baseline). Should any of these interaction terms reveal any effect, we will conduct the two subgroup analyses below. As we have not powered the trial to consider subgroups, these analyses are considered exploratory. The two subgroup analyses are:

- Site: This analysis will examine whether probiotic and egg OIT has differential effects for patients in Melbourne versus patients in Singapore.
- Age: This analysis will examine whether probiotic and egg OIT has differential effects for patients aged 5–15 years old versus patients aged 16–30.

A per-protocol analysis will also be performed whereby participants will be excluded if they completed less than 62 weeks of study treatment, are recorded to have intake of probiotic supplements or products containing the probiotic *L. rhamnosus* ATCC 53103 on 30 or more days of the active treatment period, are recorded to have intake of egg (excluding baked egg and the study OIT/Placebo dosing) on 30 or more days of the active treatment period and do not have the primary outcome data available.

The full details of statistical analyses will be specified in a separate statistical analysis plan.

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Study oversight (data and safety monitoring)

The sponsor is responsible for monitoring the progress of the trial, protocol compliance and ensure the study is being conducted according to ethical and relevant regulatory requirements.

In this trial, an independent data and safety monitoring committee (DSMC) has been appointed to review all serious adverse and non-serious events in the whole study population. The DSMC will meet annually or more frequently if needed. The DSMC consists of a biostatistician, paediatric allergist immunologists and an allergy epidemiologist. Serious adverse events will be reported to the RCH and site specific HREC. All data reported to the DSMC will be presented according to blinded treatment groups. However, if necessary, unblinded data can be obtained by an independent statistician and only be made available to the DSMC.

During the study, the sponsor or its representatives (including an independent clinical research organisation) will make site visits to review protocol compliance and ensure the study is being conducted according to ethical and relevant regulatory requirements.

Patient and public involvement

Patients and the public were not involved in the development of this study protocol.

Ethics and dissemination

This study will be conducted with the principles of Good Clinical Practice. The Royal Children's Hospital HREC (HREC 2019.082) and National Healthcare Group Domain Specific Review Board (NHG DSRB) (2019/00029) have approved this trial. Written informed consent will be obtained for all trial participants from their parent(s) or guardians. Consent will be voluntary and free from coercion and participants are free to withdraw at any time without this affecting their future care. The confidentiality of participants will be protected at all times. Results will be published in peer-reviewed journals and disseminated via presentations at international conferences.

This paper is based on the PEAT protocol V.4, 29 April 2020

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Contributors MLKT, FO and LP-CS were involved in conception and trial design. PL, ACL, LS-YW, EHT, AS-YL and ALL contributed to trial design. PL and ACL drafted the manuscript. All authors were involved in critical revision of the article for important intellectual content. All the authors were involved in final approval of the article. FO provided statistical expertise. MLKT is the principal investigator (PI) of this study. The PI is responsible for study design; collection, management, analysis and interpretation of data; writing of the report; and the decision to submit the report for publication.

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Competing interests MLKT is a past member of Nestle Nutrition Institute Medical Advisory Board Oceania, past member of Nutricia global scientific advisory board; received speaker fees from Nestle Nutrition Institute and Abbott Nutrition; consultant to Bayer Pharmaceuticals; received research funding from Abbott Nutrition, Bayer Pharmaceuticals, Prota Therapeutics; employee of Prota Therapeutics and inventor on a patent owned by MCRI 'A method for inducing tolerance'. LP-CS has received reimbursement for speaking at conferences sponsored by Danone and Nestle and consulting for Mead Johnson and Nestle. LP-CS has received research funding from Danone. ALL has received funding from Aimmune Therapeutics.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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