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Study protocol of a multicentre, randomised, controlled trial evaluating the effectiveness of probiotic and peanut oral immunotherapy (PPOIT) in inducing desensitisation or tolerance in children with peanut allergy compared with oral immunotherapy (OIT) alone and with placebo (the PPOIT-003 study)

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ABSTRACT

Introduction Peanut allergy is the the most common cause of life-threatening food-induced anaphylaxis. There is currently no effective long-term treatment. There is a pressing need for definitive treatments that improve the quality of life and prevent fatalities. Allergen oral immunotherapy (OIT) is a promising approach, which is effective at inducing desensitisation; however, OIT has a limited ability to induce sustained unresponsiveness (SU). We have previously shown that a novel treatment comprising a combination of the probiotic *Lactobacillus rhamnosus* CGMCC 1.3724 with peanut OIT (Probiotic Peanut Oral ImmunoTherapy (PPOIT)) is highly effective at inducing SU, with benefit persisting to 4 years after treatment cessation in the majority of initial treatment responders. Here we describe the protocol for a Phase IIb multicentre, double-blind, randomised, controlled trial (PPOIT-003) with dual primary objectives to evaluate the effectiveness of PPOIT at inducing SU (assessed at 8 weeks after treatment cessation) compared with placebo treatment and peanut OIT alone, in children with peanut allergy.

Methods and analysis 200 children 1 to 10 years of age with current peanut allergy confirmed by failed double-blind placebo-controlled food challenge (DBPCFC) at study screening will be recruited from three tertiary paediatric hospitals in Australia. There are three intervention arms—PPOIT, peanut OIT alone or placebo. Interventions are administered once daily for 18 months. The dual primary outcomes are: (1) the proportion of children who attain 8-week SU in the PPOIT group versus placebo group and (2) the proportion of children who attain 8-week SU in the PPOIT group versus OIT group.

Ethics and dissemination This study has been approved by the Human Research Ethics Committees at the Royal Children's Hospital (HREC 35246) and the Child and Adolescent Health Service (RGS 2543). Results will be

Strengths and limitations of this study

- This is the first double-blind placebo-controlled randomised trial to examine the effectiveness of probiotic and peanut oral immunotherapy (Probiotic Peanut Oral ImmunoTherapy (PPOIT)) in inducing desensitisation or sustained unresponsiveness (SU) in children with peanut allergy compared with oral immunotherapy (OIT) alone.
- All participants will undergo a double-blindplacebo-controlled food challenge at study entry to confirm diagnosis of peanut allergy.
- Primary outcome of SU is assessed after 8 weeks of peanut elimination, longer than most published trials of OIT.
- Monitoring of patients in the post-treatment phase will provide new information on safety events associated with SU (with ad libitum peanut intake), desensitisation (with daily peanut dosing) and with allergen avoidance.
- Measurement of peanut skin prick test, peanut and peanut component specific IgE and specific IgG4 at study entry, end of treatment, 8 weeks post-treatment and 12 months post-treatment will provide information on immune changes associated with PPOIT or OIT and also with SU, desensitisation or allergy.

published in peer-reviewed journals and disseminated via presentations at international conferences.

Trial registration number ACTRN12616000322437.

INTRODUCTION

Background

The prevalence of food allergy in the paediatric population has risen exponentially in recent decades. Now affecting 8%[1] of children and 10% of infants,[2] food allergy is a significant public health problem in Western countries.[3 4] Peanut allergy affects 2% of children and is usually lifelong.[5–7] Moreover, peanut is one of the the most common causes of anaphylaxis[8] and death[9–11] from food allergy.

There is currently no effective long-term treatment to modify the natural history of food allergy. Management involves avoidance of the food concerned, provision of an epinephrine autoinjector in some cases, and early recognition of allergic symptoms and initiation of appropriate emergency treatment. Accidental ingestion is common, causing frequent and sometimes severe reactions.[12 13] Unpredictability of symptoms from unintentional ingestion cause significant psychological distress and reduced health-related quality of life for patients and their families.[14] The ultimate goal is to develop immunomodulatory strategies that target the underlying immune dysregulation of allergic disease to induce a tolerant state, allowing allergic individuals to safely ingest foods on a lasting basis.

Although the precise mechanisms are incompletely understood, in broad terms, the development and maintenance of oral tolerance appears to be an active and antigen-specific immune response that depends on both: (a) exposure to allergen which induces an allergen-specific immune response and (b) initial allergen presentation in an ambient immunological milieu which optimally favours regulatory immune responses. It is therefore logical to consider both elements in therapeutic and preventive strategies. This is the basis of our treatment approach which comprises administration of both allergen (peanut oral immunotherapy (OIT)) and an adjunctive immune response modifier (IRM), as discussed further below. So far, most immunotherapy studies have focussed on food allergen delivery alone, without associated IRM.[15 16]

Tolerance can be defined as the permanent state of immune unresponsiveness to food antigens that does not require any continuing food exposure.[17] Since assessing for permanent 'tolerance' is difficult within the time frame of clinical trials,[18] the ability to induce 'sustained unresponsiveness (SU)' is used as a surrogate measure of more prolonged protection in food immunotherapy studies. SU is defined as the ability to tolerate a food after a period of avoidance of at least weeks or months and is considered to reflect redirection of the underlying allergic response towards tolerance. SU is optimally measured by double-blind placebo-controlled food challenge (DBPCFC), with a cumulative dose of peanut protein equivalent to the amount required for diagnosis or exclusion of peanut allergy in the clinical setting, performed at least several weeks after the cessation of treatment.[19] Another outcome that can be achieved with food immunotherapy is desensitisation, defined as a transient increase in reaction threshold that is only maintained with continuing and regular allergen exposure. Desensitisation reflects downregulation of effector cell/mast cell activation without redirection of the underlying allergic response.

OIT has garnered intense interest as a potential food allergy treatment. OIT has been shown to consistently induce desensitisation in a high proportion of participants; however, effectiveness of OIT to induce SU appears to be modest.[15 20] Few studies have examined for SU following peanut OIT. The first uncontrolled open-label study of peanut OIT (maintenance dose 125 mg peanut protein for 22 months) reported SU in 13% (3/23) of treated children.[21] A second uncontrolled study (4 g peanut protein for 5 years) reported SU in 31% (12/39) of OIT-treated patients by intention-to-treat analysis (ITT) (or 50% (12/24) by per protocol analysis).[18] As there was no control group in either of these studies, it is difficult to ascertain the benefit from OIT over and above natural resolution which can occur in ~20% of children within 5 years.[5 22] A third open-label controlled study of peanut OIT (4 g peanut protein for 2 years) in 43 patients 5 to 45 years of age reported SU in 30.4% (7/23) of the OIT group compared with 0% (0/20) of control patients.[23] The only double-blind, randomised, placebo-controlled trial of peanut OIT[24] that evaluated SU as an outcome randomised 120 peanut allergic patients (aged 7 to 55 years) to receive: (1) peanut OIT (maintenance 4 g) for 104 weeks followed by peanut discontinuation (peanut-0, n=60), (2) peanut OIT (maintenance 4 g) for 104 weeks followed by 300 mg peanut daily (peanut-300, n=35) or (3) oat flour for 104 weeks followed by peanut avoidance (placebo group, n=25). All participants were followed for 3 years (156 weeks). DBPCFC was performed at week 104, 117, 130, 143 and 156. The rate of SU (defined as those passing both the week 104 and 117 challenges) in the peanut-0 group was 35% (21/60), compared with 4% in the placebo group.[24] These studies show that while OIT can induce SU in some patients, effectiveness appears to be limited.

Patients with 'desensitisation' (without evidence of SU) remain at risk of allergic reactions to allergen, including to previously tolerated doses, despite continuing with regular allergen exposure. Studies indicate that children who have been desensitised following OIT experience allergic reactions, including anaphylaxis requiring epinephrine treatment, more frequently than if they had continued with traditional food avoidance management strategies.[25–28] This highlights the importance of identifying an effective immunomodulatory therapy that can induce SU or tolerance, which are expected to provide greater benefit and safety to individuals with food allergy than is achieved by desensitisation.

We postulated that IRM may enhance the ability of allergen OIT in inducing SU and tolerance. IRM are a class of tolerogenic compounds, typically of microbial origin, which modulate immune responses by acting on antigen-presenting cells through pattern recognition receptors, including Toll-like receptors. One well-characterised IRM is the probiotic bacteria *Lactobacillus rhamnosus* GG, with demonstrated immunomodulatory effects in vitro and in vivo that can support acquisition of oral tolerance, including induction of T regulatory and Th1 cytokine responses.[29–32] Furthermore, oral co-administration of *L. rhamnosus* GG with antigen has been shown to enhance antigen-specific IgA responses, which are also known to promote oral tolerance.[33 34]

In a landmark forerunner study to the current trial, we reported the first randomised controlled trial (RCT)[35] evaluating a combination of probiotic *L. rhamnosus* CGMCC 1.3724 (which is genetically indistinguishable from *L. rhamnosus* GG) together with probiotic and peanut OIT (Probiotic Peanut Oral ImmunoTherapy (PPOIT)).[35] Sixty-two children with peanut allergy were randomised to receive either PPOIT or placebo for 18 months. The probiotic *L. rhamnosus* CGMCC 1.3724 was administered as a fixed daily dose (2×10^{10} cfu), while the peanut OIT was a daily dose of peanut protein starting at low doses and increasing to a maintenance dose of 2 g peanut protein. SU was assessed by DBPCFC performed 4 (±2) weeks after cessation of treatment. Eighty-two per cent of PPOIT-treated participants achieved SU to peanut compared with only 3.6% of placebo-treated children. PPOIT treatment was also associated with reduced peanut SPT and specific IgE (sIgE), and increased peanut specific IgG4 (sIgG4), suggesting modulation of the underlying allergic response to peanut. Furthermore, the clinical benefit of PPOIT was shown to be long-lasting, with 70% of PPOIT-treated children who achieved SU at the end of treatment still having challenge-confirmed SU at 4 years after end of treatment (assessed by DBPCFC performed following 8 weeks of peanut elimination).[36] For logistic reasons, this preliminary study did not compare PPOIT with OIT alone. Having demonstrated that PPOIT is highly effective at inducing long-lasting SU in children with peanut allergy, it is now imperative to examine whether the combined PPOIT treatment is more effective than peanut OIT at inducing SU, and hence to determine if the probiotic IRM does indeed offer added benefit over and above peanut OIT alone. It is also necessary to confirm the previous findings in a larger multicentre randomised trial.

This paper reports the research protocol for a Phase IIb multicentre, double-blind placebo-controlled randomised trial evaluating the efficacy of PPOIT at inducing SU compared with both placebo and peanut OIT alone.

AIMS

Primary objectives

1. To compare the proportion of children who attain 8-week SU in PPOIT and placebo-treated groups.
2. To compare the proportion of children who attain 8-week SU in PPOIT and OIT-treated groups.

Secondary objectives

1. To compare the proportion of children who attain 8-week SU (passed T1 and T2 challenges) in OIT and placebo-treated groups.
2. To compare the proportion of children who achieve full desensitisation (passed T1 challenge) at the end of treatment in (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
3. To compare the total cumulative dose of peanut protein tolerated during the end-of-treatment T1 challenge in (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo. This will determine the total dose tolerated in partially desensitised (those who did not achieve full desensitisation) and allergic participants.
4. To compare the proportion of children who are eating peanut in their diet 12 months after end of treatment in (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
5. To compare peanut skin prick test (SPT) and peanut and peanut component sIgE and sIgG4 levels at the end of treatment, and at 8 weeks and 12 months after treatment in PPOIT, OIT and placebo groups; and to examine their correlation with SU.
6. To evaluate the safety and tolerability of PPOIT.

Exploratory objectives

1. To compare quality of life at the end of treatment and 12 months later in PPOIT, OIT and placebo groups.
2. To determine cost-effectiveness of PPOIT compared with OIT and placebo in terms of achieving SU, full desensitisation and quality of life changes at 12 months after treatment.

METHODS AND ANALYSIS

Study design

This is a three-armed, multicentre, randomised (2:2:1), stratified (by study site, age and SPT), blinded, placebo-controlled, parallel-group superiority trial

- PPOIT=Probiotic and peanut OIT taken daily for 18 months.
- OIT=Probiotic placebo and peanut OIT taken daily for 18 months.
- Placebo=Probiotic placebo and OIT placebo taken daily for 18 months.

Study setting

This is a multicentre study conducted in three children's hospitals in Australia—the Royal Children's Hospital (RCH) in Melbourne, Perth Children's Hospital (PCH) in Nedlands, and the Women's and Children's Hospital (WCH) in Adelaide. Participants recruited from allergy departments of these tertiary hospitals and from the general community reached by the media.

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, OIT and management of allergic reactions. Interim doses of OIT will be administered at home.

All participants will be provided with an anaphylaxis action plan and an EpiPen/Epipen Jr, and educated in the management of allergic reactions (standard care for peanut 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

Two hundred children, 1 to 10 years of age, with current peanut allergy confirmed by DBPCFC at study screening. Participants will be randomised to PPOIT (n=80), OIT (n=80) or placebo (n=40).

Participant eligibility was established prior to enrolment and randomisation.

Inclusion criteria

Participants were eligible for the study if they met all the following criteria:

- Children aged between 1 and 10 years.
- >7 kg (the weight considered safe for the administration of an EpiPen Jr).

Confirmed diagnosis of peanut allergy as defined by a failed DBPCFC with peanut and a positive SPT or sIgE to peanut at screening visit (a positive SPT is defined as weal size ≥3 mm and a positive sIgE is defined as >0.35 kUA/L).

Exclusion criteria

Participants were not eligible for the study if they met 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 doses of intramuscular epinephrine or an intravenous epinephrine infusion for management of an allergic reaction).
- Severe anaphylaxis during the study entry DBPCFC.
- Forced expiratory volume in 1 second (FEV_1) <85% predicted at rest and FEV_1/FVC (forced vital capacity) ≤85% at rest or ongoing chronic persistent asthma (as per National 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, immune-compromised 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 within the past 6 months (does not include formula).
- 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 patient's ability to participate in the study; for example, increased risk to the participant.
- Patients 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).

Patient recruitment, study procedure and data collection

The start date of the trial was 4 July 2016 and the planned end date is October 2020.

Consent procedure

Participants who are identified as potentially being suitable to participate in the study and their parents will be sent an Information Statement and Consent Form as approved by the RCH and local site Human Research Ethics Committee (HREC) and Research Governance Office.

Prior to full study enrolment and gathering any further study-specific personal information or performing any study-specific procedures (eg, screening DBPCFC), a signed consent form will be obtained from the parent(s) or guardian of the participant. Randomisation and enrolment will take place up to 1 week prior to the Rush Induction visit (initiation of study treatment). The Rush Induction visit may be delayed up to 3 months after screening visit.

Randomisation and concealment mechanism

Participants will be enrolled and randomised up to 1 week prior to Rush Induction, and within 3 months of their screening appointment. Randomisation will be to PPOIT, OIT or placebo groups, with an allocation ratio of 2:2:1. Randomisation will be stratified by study site (RCH, PCH and WCH), by age (1 to 5 years; 6 to 10 years) and by peanut SPT weal size (≤10 mm; >10 mm). Stratification by age and SPT weal size is necessary for data analysis because younger age and smaller SPT size are associated with a greater likelihood of natural resolution. Each study site will have their own randomisation list stratified by age and peanut SPT weal size. Randomisation will be in randomly permuted blocks of variable length. An independent statistician in the Clinical Epidemiology and Biostatistics Unit (CEBU) at the Murdoch Children's Research Institute (MCRI) will provide the randomisation schedules to hospital pharmacies at each site.

Participant eligibility will be established prior to enrolment and randomisation. A unique participant screening number will be allocated to each consenting 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—a small number of participants who are eligible for the study at study screening may decide not to proceed with the study between the challenge and the Rush Induction day.

The pharmacist 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 on the participant's CRF (case report form). The pharmacist will prepare the participant's allocated study treatment and label the treatment with the participant's randomisation number. Participants, outcome assessors, other research staff, treating clinicians, investigators and statistical analyst will be blinded to treatment allocation.

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

Rush induction visit (T0)—day 1

In this phase, participants will receive a single dose of 2×10^{10} cfu *L. rhamnosus* GG or placebo followed by increasing doses of peanut (or placebo) OIT, to reach a final dose of 12 mg of peanut protein or placebo (cumulative dose 24 mg peanut protein or placebo).

Participants who complete the Rush protocol without reaction will commence the Buildup Phase at a daily dose of 25 mg peanut protein or placebo 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 peanut OIT (or placebo OIT) will be increased every 2 weeks until a maintenance dose of 2000 mg 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* GG 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 2 g peanut protein or placebo and a daily dose of 2×10^{10} cfu *L. rhamnosus* GG or placebo until a total of 18 months of treatment is completed.

Clinical endpoints

Primary endpoint

Sustained unresponsiveness—time point T2

SU 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 desensitisation DBPCFC at the T1 visit will proceed to the SU DBPCFC during the T2 visit. SU is defined as passing both the T1 and T2 DBPCFCs.

Secondary endpoints

Desensitisation—time point T1

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

Peanut intake and reactions—time point T3

Peanut intake and reactions to peanut will be recorded in the participant's diary during the period between T1 and T3. Participant/parents will be given a 'Follow-up Diary' at T1 (if participant fails T1 DBPCFC) or at T2 (if participant passes T1 DBPCFC) to record reactions to peanut or other food products, and also peanut consumption. Participants are provided with instructions for peanut ingestion/avoidance based on their treatment outcomes: (1) participants who achieve SU at the end of treatment will be instructed to incorporate peanut into their diet ad libitum, (2) participants who achieve desensitisation without SU will be instructed to commence a daily ingestion of 1 to 2 peanuts while maintaining avoidance of all other peanut intake, and (3) participants who remain allergic to peanut will be advised to continue strict avoidance of peanut in their diet. Participants/parents will maintain the diary from T1/T2 to T3 and data will be collected from the participant's diary at the T3 visit.

Study outcomes

Primary outcomes

- Proportion of participants with 8-week SU (passed T1 and T2 challenges) in PPOIT versus placebo.
- Proportion of participants with 8-week SU (passed T1 and T2 challenges) in PPOIT versus peanut OIT.

Secondary outcomes

- Proportion of participants with 8-week SU (passed T1 and T2 challenges) in peanut OIT versus placebo.
- Proportion of participants who achieve full desensitisation (passed T1 DBPCFC) in PPOIT versus placebo, PPOIT versus OIT, and OIT versus placebo.
- The cumulative dose tolerated during the T1 challenge—determined by performing a DBPCFC—(cumulative doses below the reaction-eliciting dose if there is a reaction; or total cumulative challenge dose if there is no reaction) in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Proportion of participants who are eating peanut in their diet without reaction at 12 months after the cessation of treatment in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Change in peanut SPT weal size at end of treatment, and 8 weeks and 12 months after the end of treatment

in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Change in immunological measures (sIgE and sIgG4) at the end of treatment, and 8 weeks and 12 months after the end of treatment in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Correlation between change in peanut SPT weal size and SU at the end of treatment, and 8 weeks and 12 months after the end of treatment in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Correlation between change in immunological measures (sIgE and sIgG4) and SU at the end of treatment, and 8 weeks and 12 months after the end of treatment in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Incidence and severity of treatment-emergent adverse events in PPOIT, OIT and placebo groups.
- Peanut intake (accidental or intentional) from the end of treatment to 12 months post-treatment.
- Reactions to peanut from the end of treatment to 12 months post-treatment.

Exploratory outcomes
- Quality of life score at the end of treatment and at 12 months after the end of treatment in: (1) PPOIT versus placebo; (2) PPOIT versus OIT; and (3) OIT versus placebo.
- Cost per case of SU, full desensitisation and quality-adjusted life year gained at 12 months post-treatment.

Study outline described in figure 1.

Study visits

T0 visit

The following assessments will be conducted at the day 1 Rush Induction visit (T0):
- Allergy questionnaire.
- Vital signs (blood pressure*, pulse, respiration and temperature).
- Spirometry (all children over 8 years).
- Weight/height.

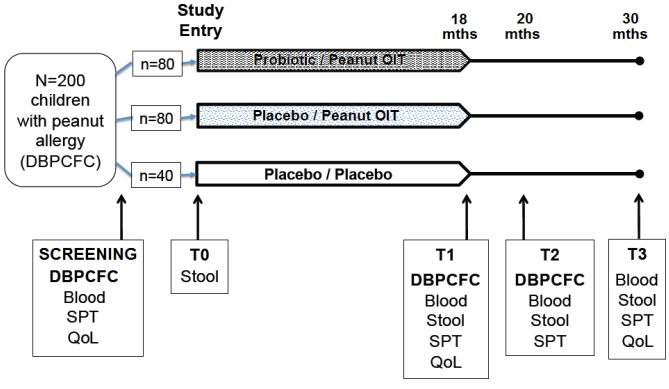

Figure 1 Study outline. DBPCFC, double-blind placebo-controlled food challenge; mths, months; OIT, oral immunotherapy; QoL, quality of life questionnaire; SPT, skin prick test.

- Faecal sample (collected by parents at home).
- Anaphylaxis education.
- Dispense participant diary.
- Anaphylaxis action plan and EpiPen prescribed.
- Dispense OIT.

Buildup phase visits

The following assessments will be conducted at these visits:
- Allergy questionnaire.
- Vital signs (blood pressure*, pulse, respiration and temperature).
- Weight/height.
- Anaphylaxis education.
- Review participant diary.
- Collect study treatments for review of compliance.
- Dispense OIT.
- Review adverse events and concomitant medications.

Maintenance phase visits

The following assessments will be conducted at these visits:
- Allergy questionnaire.
- Vital signs (blood pressure*, pulse, respiration and temperature).
- Weight/height.
- Anaphylaxis education.
- Review participant diary.
- Provide faecal collection pot and instructions (for collection at T1).
- Collect study treatments for review of compliance.
- Dispense OIT.
- Review adverse events and concomitant medications.

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 history.
- Allergy questionnaire.
- Vital signs (blood pressure*, pulse, respiration and temperature).
- Spirometry (all children over 8 years).
- Weight/height.
- Parent(s) to complete Food Allergy Quality of Life-Parent Form (FAQL-PF).
- SPT (peanut, egg, milk, cashew, almond, pistachio, hazelnut, dust mite, and positive and negative control).
- Anaphylaxis education.
- Review participant diary.
- Collect study diary and dispense new diary once allergic status known.
- Collect study treatments for review of compliance.
- Review adverse events and concomitant medications.
- DBPCFC.
- Blood and faecal sample (faecal sample collected by parents at home).
- Provide faecal collection pot and instructions (for collection at T2).

T2 visit

There will be a visit at 20 months (T2, 8 weeks after T1) for assessment of SU.

The following assessments will be conducted at this visit:

- Medical history.
- Allergy questionnaire.
- Vital signs (blood pressure*, pulse, respiration and temperature).
- Spirometry (all children over 8 years) (only if they pass T1 DBPCFC).
- Weight/height.
- SPT (peanut, egg, milk, cashew, almond, pistachio, hazelnut, dust mite, and positive and negative control).
- Anaphylaxis education.
- Review participant diary.
- Collect study diary and dispense new diary once allergic status known.
- Review adverse events and concomitant medications.
- DBPCFC (only if they pass T1 DBPCFC).
- Blood and faecal sample (faecal sample collected by parents at home).

*For participants ≤3 years of age, blood pressure readings will be at the medical staff's discretion.

6 months post-treatment phone call

The following assessments will be conducted:

- Allergy questionnaire.

T3 visit

The T3 visit will be performed 12 months after the end of treatment (T1). During this visit, peanut ingestion and reactions to peanut in the 12-month period since the end of treatment will be recorded.

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 and at least 24 hours apart. The cumulative amount of peanut or placebo powder administered is 9.9 g (equivalent to 4950 mg of peanut protein) (table 1). The doses will be administered at 15 min intervals, if the participant has not had a reaction consistent with a predefined stopping criteria (box 1) to the previous dose. The participant will be observed for a minimum of 2 hours following food challenge and will be discharged home if no adverse reactions are noted.

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 peanut component and no reaction to the placebo component (pharmacy will only un-blind the contents of part A and B after both parts are completed and provided the participant has failed one part and not the other part of the DBPCFC).
- 'Passed' if both part A and B of the challenge are completed without reaction. The contents of part A and B are not be un-blinded.
- 'Inconclusive' if participant reacts to both part A and B (contents of part A and B will not be un-blinded) 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-National Institute of Allergy and Infectious Diseases (NIH NIAID) Consortium for Food Allergy Research-specific grading system for allergic reactions.[37]

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 angio-oedema.
- Vomiting (excluding gag reflex) and/or diarrhoea.
- Persistent cough (ie, not just intermittent and transient throat clearing), wheeze (either audible (without stethoscope) or on auscultation with stethoscope), change in voice, stridor, difficulty breathing and long bursts of sneezing/persistent rhinorrhoea (persistent defined as on three or more doses or more than 40 min).
- Collapse, hypotension (ViCTOR chart).

Table 1 Food challenge doses

Dose	Dose, mg (peanut protein)	Weight of flour, mg	Cumulative dose, mg (peanut protein)
1	80	160	80
2	160	320	240
3	320	640	560
4	640	1280	1200
5	1250	2500	2450
6	2500	5000	4950

ViCTOR, Victorian Children's Tool for Observation and Response.

Skin prick test and laboratory tests

At the time of the screening visit as well as the end of treatment (T1), and at 8 weeks (T2) and 12 months (T3) after end of treatment, up to 20 mL of blood will be collected for the measurement of sIgE and sIgG4 against whole peanut and peanut components (Ara h 1, Ara h 2 and Ara h 3) by ImmunoCAP (Phadia AB, Uppsala, Sweden). Plasma and peripheral blood mononuclear cells will be isolated and stored at −80°C or in liquid nitrogen for exploratory immunological studies.

SPT for peanut, egg, milk, cashew, almond, pistachio, hazelnut, dust mite, 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 parent diary records as well as by treatment capsule/tub counts and weighing of returned bottle contents.

Adverse events reporting

Adverse events will be recorded from signed consent until 8 weeks after the last dose of study product in the participants' diary. 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 200 participants, randomly allocated in a 2:2:1 ratio to PPOIT (n=80), OIT (n=80) and placebo (n=40). Since the study aims to assess two primary treatment comparisons, a hierarchical 'fixed-sequence' testing strategy will be used to maintain a global type I error of 5% for the study.

For the first treatment comparison, PPOIT versus placebo, we conservatively estimate a proportion of 60% of participants will achieve SU with PPOIT based on the results of our previous RCT;[35] and the natural rate of resolution of peanut allergy is conservatively assumed to be equal to or below 15%.[35] Applying a 2:1 ratio for randomisation to PPOIT and placebo arms, sample sizes of n=70 and n=35 will provide >99% power with two-tailed 0.05 significance level for the Pearson χ^2 test to detect the difference between 60% SU in the PPOIT group and 15% in the placebo group.

For the second treatment comparison, PPOIT versus OIT, a pilot study of peanut OIT reported SU in 30% of participants.[23] A sample size of n=70 in PPOIT and n=70 in OIT groups will provide 85% power with two-tailed 0.05 significance level to detect the difference between 60% SU in the PPOIT group and 35% in the OIT group.

Allowing for a 12.5% loss to follow-up, we will recruit 200 children, resulting in approximately 80 children in each of the PPOIT and OIT groups, and 40 in the placebo group. In our completed RCT, loss to follow-up was 10%.

Statistical analysis

Data handling, verification and analysis will be performed by an independent clinical research organisation, Datapharm Australia, in collaboration with the CEBU at MCRI. Statistical analysis will follow standard methods for randomised trials and the primary analysis will be by ITT.

All available data from all participants who received any investigational product will be included in the analysis of the safety data.

All demographic and baseline continuous outcomes will be presented as mean and standard deviation (or medians and IQRs for skewed data), while categorical outcomes will be presented as absolute and relative frequencies in the three groups.

Unless specified otherwise, statistical tests will be conducted at the 5% significance level (alpha) and all CIs will be reported as 95%.

The primary analysis will be conducted when all participants have either completed the T2 visit or terminated the study prior to their T2 DBPCFC. At the completion of the last T2 visit, database lock and statistical analyses on the primary and secondary outcomes to the T2 time point will be conducted by an independent unblinded statistician (Datapharm Australia). Participants and study staff will remain blinded to treatment allocation and will continue onto the Monitoring (non-treatment) phase. When all participants have either completed the T3 visit or terminated the study prior to T3, the final database lock will occur and statistical analyses on the secondary outcomes to T3 time point and exploratory outcomes will be performed.

Primary outcomes

The primary endpoint is whether a participant has SU (passed T1 and T2 challenges). Results will be summarised as the number and proportion of participants with SU in the three treatment groups. Comparison between PPOIT with placebo as well as between PPOIT with OIT will be presented as risk differences and relative risks, accompanied by their 95% CI, with the null hypothesis of no difference between the groups tested using Pearson χ^2 statistic. Moreover, logistic regression analysis with adjustment for the stratification variables (centre, age category and SPT weal size category) used in the randomisation, will be conducted and ORs with respective CIs reported.

Secondary outcomes

Group comparisons (PPOIT versus placebo; PPOIT versus OIT; and OIT versus placebo) regarding dichotomous outcomes will be presented as risk differences and relative risks, accompanied by their 95% CI, as well as using OR estimates with 95% CIs, obtained from a logistic regression analysis with adjustment for the stratification variables used in the randomisation.

If normally distributed, continuous outcomes will be compared using differences between mean values, estimated from linear regression models. In particular, peanut SPT weal size, sIgE and sIgG4 levels will be reported as mean and SD by treatment group, and presented at the end of

treatment (T1), at T2 and at the end of study (T3). The difference in mean between groups and the corresponding 95% CIs (PPOIT versus placebo; PPOIT versus OIT; and OIT versus placebo) will be obtained by using an unadjusted and adjusted (by stratification variables used in the randomisation) linear regression and the hypothesis of no difference between the groups tested with a t-test. Analogously, quality of life continuous outcome measures will be summarised by treatment group at the end of treatment (T1) and the end of study (T3) and presented as a difference in means between groups and the corresponding 95% CI (PPOIT versus placebo and PPOIT versus OIT), obtained by using unadjusted and adjusted linear regression. If continuous outcomes do not follow normal distributions, they will be summarised as median and IQR in the three groups, and comparison between groups will be performed by the Wilcoxon rank-sum (Mann-Whitney) test. The cumulative tolerated dose at the T1, which is inherently non-normally distributed, will be will be presented as medians and IQR by treatment groups and compared between treatment groups in pairwise fashion using the Wilcoxon rank-sum test.

A per-protocol analysis will also be performed whereby participants will be excluded if they completed less than 68 weeks of study treatment, or are recorded to have intake of probiotic supplements or products containing the probiotic *L. rhamnosus* GG on 30 or more days of the active treatment period, or are recorded to have intake of peanut on 30 or more days of the active treatment period or do not have the primary outcome data available or treatment compliance is not between 80% and 120% for both the buildup and maintenance phases of the study.

Safety tables will present the frequency and percentage of participants with an adverse event and the number of events, by relationship to study drug (unrelated, unlikely to be related, possibly related and probably related), by severity, by study phase and according to whether the event is an allergic reaction or a non-allergic reaction. Similar separate summaries of serious adverse events (SAEs) will also be provided.

Full details of the primary and secondary statistical analyses will be specified in a separate statistical analysis plan (SAP) which will be finalised before study database lock. The SAP will detail covariates to be considered in the primary analysis model as well as subgroup and sensitivity analyses to be performed. The SAP will also outline the imputation strategy to handle missing data.

Study oversight (data and safety monitoring)

The Sponsor is responsible for monitoring the progress of the trial, protocol compliance and ensuring 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 and two paediatric allergist immunologists. SAEs will be reported to the RCH and site-specific HREC.

All data reported to the DSMC will be presented according to blinded treatment groups, with treatment groups labelled as 'A', 'B' and 'C'. 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 RCH HREC (HREC 35246) and Child and Adolescent Health Service HREC 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.

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Contributors MT, FO, SP and MSG were involved in conception and trial design. MO, PQ, PL and AD contributed to trial design. ACL and PL 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. MT was principal investigator (PI) from study conception to February 2018, after which PL assumed the PI role. 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 MT is a past member of Nestle Nutrition Institute Medical Advisory Board Oceania; past member of Nutricia global scientific advisory board; speaker fees from Nestle Nutrition Institute and Abbott Nutrition; consultant to Bayer Pharmaceuticals; research funding from Abbott Nutrition, Bayer Pharmaceuticals, Prota Therapeutics; employee of Prota Therapeutics and inventor on a patent owned by Murdoch Children's Research Institute 'A method for inducing tolerance'.

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