HYPES: Peanut allergy desensitisation using sequential hypoallergenic and roasted peanuts

STUDY IDENTIFIER: HYPES

VERSION: 1

DATE: 09/01/2017

PRINCIPAL INVESTIGATOR:
Dr Tim Chataway

SPONSORING INSTITUTION:
Flinders Medical Centre
Flinders Drive, Bedford Park SA 5042, AUSTRALIA

Australian and New Zealand Clinical Trials Registry:
ACTRN12617000803392

Ethics Approval:
Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC EC00188)

PROTOCOL AMENDMENTS

Version	Date of HREC Approval	Amendments

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007 – updated May 2015) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

I agree that the study will be conducted in accordance with the conditions outlined in the protocol (subject to any amendments). I have read and understood the protocol.

I understand that the information in this protocol is confidential. Publication of information related to this protocol in formats including, but not limited to, conference abstracts, posters or presentations; seminars, journal articles, public reports and internet postings, must be submitted to the Study Steering Committee for consideration. Proposals for said activities must be within a reasonable time frame of any due dates. Approval for all said activities must have the written permission of the Chair of the Steering Committee or their delegate prior to the event.

Investigator's Name:
Dr Billy Tao

Investigator's Signature:

Date: 09/01/2017

Study Site: Allergy SA

COORDINATING CENTRE:

Chair Steering Committee:
Dr Tim CHATAWAY
Flinders University

Chair Steering Committee signature:

Date: 09/01/2017

GLOSSARY OF ABBREVIATIONS

AI	Associate Investigator
AE	Adverse Event
CI	Chief Investigator
CRF	Case Report Form
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HREC	Human Research Ethics Committee
NHMRC	National Health and Medical Research Council
OIT	Oral Immunotherapy
SAE	Serious Adverse Event
SPT	Skin Prick Test
TGA	Therapeutic Goods Association

Contents

1. INVESTIGATORS AND FACILITIES

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1.2. Study Locations

Allergy SA

Beulah Park SA 5067 AUSTRALIA

Flinders Medical Centre

Bedford Park SA 5042 AUSTRALIA

2. STUDY MANAGEMENT

Flinders University is the nominated sponsor for the trial.

The Principal Investigator at each study centre will be responsible for the conduct of the study at their centre including informed consent, recruitment, data collection and maintenance of study documentation. Handling of investigational products will be the responsibility of clinical trial staff.

The Coordinating Centre Steering Committee, chaired by Dr Tim Chataway, will provide direct day-to-day management for the trial.

The core Steering Committee will meet regularly (at least monthly).

3. SERIOUS ADVERSE EVENT AND TRIAL MONITORING COMMITTEES

3.1. Serious Adverse Event Committee

A Serious Adverse Event (SAE) Committee will review all participant SAEs to determine whether there is any likelihood that involvement in the trial could have contributed. Cause of death will be determined from autopsy results or other hospital summaries by relevant medical personnel. This committee will meet three-monthly (or as required).

3.2. Data and Safety Monitoring Committee

An independent Data and Safety Monitoring Committee (DSMC) will be set up to review the yearly progress of the trial and provide feedback to the Steering Committee. The DSMC will review general study progress (recruitment, compliance, loss to follow-up, adverse events). The DSMC will also provide advice regarding external issues that may impact on the study (for example changes in clinical practice). The DSMC will review all SAEs. This committee will meet six-monthly or as required.

4. FUNDING

This study is supported by funding received from the Channel 7 Children's Research Foundation, South Australia, Australia.

5. INTRODUCTION AND BACKGROUND

5.1. Introduction

Peanut allergy affects up to 3% of Australian children (Osborne et al., 2011) and the prevalence rate is rising (Mullins et al., 2009, Sicherer et al., 2010). Most affected children will end up as peanut-allergic adults because less than 20% outgrow their allergic status over a 10-year period (Byrne et al., 2010).

Currently there is no safe method for treatment of peanut allergy. Management is simply avoidance and an action plan, which includes the prescription of an adrenaline self-injecting device (Epipen®) for those at high risk. Such an approach is far from ideal, and does not improve the quality of life of affected children and their families (Avery et al., 2003, Primeau et al., 2000, Bollinger et al., 2006). Further, avoidance cannot be guaranteed and accidental ingestion may be dangerous or even fatal (Boyce et al., 2010).

Oral immunotherapy (OIT) is a progressive desensitisation method giving patients increasing doses of an allergen until a target is reached over time. It is important to note that desensitisation can only provide temporary protection. If regular ingestion is discontinued the original allergy frequently returns. Consequently, patients need to continue ingesting the allergen regularly and possibly indefinitely.

This kind of approach has been extensively studied in the treatment of cow's milk, egg and peanut allergies (Nwaru et al., 2014). For peanut allergy, OIT using roasted peanut products (Hofmann et al., 2009, Clark et al., 2009, Jones et al., 2009, Blumchen et al., 2010, Varshney et al., 2011, Anagnostou et al., 2011, Anagnostou et al., 2014, Tang et al., 2015) showed early promise but further progress was hampered by the occurrence of frequent treatment-related adverse events (45-93%) and high rates of withdrawal (up to 35%).

OIT in its current form is not considered sufficiently safe for routine clinical use (Thyagarajan et al., 2010). As an illustration, Blumchen et al 2010 recorded 185 adverse events in 23 subjects receiving OIT, with 9/23 (39%) withdrawing. This figure did not include "subjective complaints" including oral-pharyngeal itchiness or abdominal pains. A recent Australian study by Tang et al 2015 demonstrated the lowest incidence of adverse events to date, but at 45% is still unacceptably high.

All published studies to date have required hospital involvement because of the high risk of adverse events. Subjects would need at minimum hospital-based outpatient supervision for up-dosing administration, and in a large number of cases hospital inpatient admission for OIT initiation. Such reliance carries substantial cost implications, adds further stress to our already

strained hospital resources, and creates insufficient capacity to provide OIT to all patients who would significantly benefit from it. There is urgent need for research focusing on the safe and efficacious administration of OIT that can occur in a community setting, representing a paradigm shift in the management of peanut allergy.

5.2. Study Rationale

In 2001 Beyer et al (Beyer et al., 2001) observed that the prevalence of peanut allergy in China was lower than Western countries and hypothesised that this was because peanuts consumed there were either boiled or fried rather than roasted. They demonstrated that boiling peanuts for 20 minutes was able to reduce IgE reactivity when compared to roasted peanuts. More recent publications have confirmed the reduction of IgE-reactivity as a consequence of boiling (Mondoulet et al., 2005, Maleki et al., 2010, Cabanillas et al., 2012, Kim et al., 2013), but none has investigated the effect of boiling longer than 60 minutes.

Our new OIT treatment is an extension of ideas from research undertaken as part of a previously funded Channel 7 Children's Research Foundation Grant (Reference number 14885) and is based on **two postulates**. The **first** is that a hypoallergenic peanut will result in fewer adverse events when compared to raw/roasted peanuts. Hypoallergenic forms of foods have been trialled in desensitisation of milk and egg allergies (mainly as baked products) (Nowak-Wegrzyn et al., 2008, Lemon-Mule et al., 2008, Turner et al., 2013), but not yet in peanut allergy. We have experimentally determined the boiling time required to produce hypoallergenic peanuts by boiling peanuts for up to 12 hours and analysing them with western blot, inhibition ELISA, skin prick test, mass spectrometry and flow cytometry (Tao et al., 2016). This paper by Tao et al is the first to establish the science of desensitisation using boiled peanuts. We found:

1. Extended boiling progressively reduced peanut allergenicity through a combination of leaching of allergens into cooking water, fragmentation of allergens, and denaturation of conformational epitopes.

2. 2-hour boiling led to an 8-fold reduction in IgE-binding capacity of boiled peanuts while 12-hour boiling led to a 19-fold reduction. Mass spectrometry revealed an increasing number of unique allergen peptides with longer boiling times (42-fold more in 12-hour, 5-fold more in 2-hour boiled than raw peanuts), while raw, 2-hour and 12-hour boiled peanuts were equivalent in their ability to stimulate T cell activation and proliferation.

We concluded that boiling progressively reduces IgE-reactivity without affecting T cell reactivity (which may be a prerequisite for desensitisation), making boiled peanut a suitable candidate for oral immunotherapy.

The **second** postulate is that boiled peanuts alone may not be able to fully desensitise peanut-allergic patients as they don't contain a complete repertoire of allergen epitopes, so a second OIT phase using roasted peanuts is required. Introducing boiled peanut in the first phase of OIT is predicted to reduce adverse events because boiled peanuts are hypoallergenic. Completion of a first phase of OIT using boiled peanut is also predicted to reduce adverse events in phase 2 because patients are at least partially desensitised. Therefore, the combination of boiled and roasted peanuts should provide full desensitisation with reduced adverse events.

We followed these ideas with a pilot feasibility study testing whether this novel 2-step desensitisation strategy can be safely carried out in the community setting independent of hospital involvement. It also facilitated further improvements to the original protocol and formed the basis of this proposal for a larger study.

The original pilot study utilised 2-hour boiled peanuts for partial desensitisation in phase 1 (7 months), followed by roasted peanuts for full desensitisation in phase 2 (5 months). After Phase 2, all patients were challenged with 10 roasted peanuts to prove that they were indeed desensitised. All up-dosing steps were carried out at patients' homes, and subjects were only required to visit Allergy SA for outpatient review at the first dose of boiled peanut (phase 1), the first dose of roasted peanut (phase 2), and the final 10-roasted-peanut challenge test.

We have preliminary data on a total of 12 children who have undergone OIT, all with clear peanut-allergic histories, positive skin tests, and a failed oral food challenge. At completion, all 12 (100%) children were able to ingest 10 roasted peanuts in one bolus dose with no reaction, demonstrating that desensitisation has been achieved. We expect to publish these results later this year.

In phase 1 (boiled peanut) three children recorded mild adverse events, providing an adverse event rate of 25% (95%CI 0.5-49.5%). Adverse events all occurred at the start of phase, and all resolved when the starting dose was reduced from ¼ 2-hour boiled peanut to 1/16 and then gradually increased back to 1/4 over 2 weeks.

In phase 2, of the 12 subjects who ingested roasted peanuts progressively, only 2 reported a single adverse event: one with mild abdominal pain and a small vomit, and the other with a feeling of "oral puffiness" but no visible swelling, both occurring after ingesting ¼-peanut at start of phase. As OIT progressed they were able to tolerate with ease all dose increments carried out at home without any problem. This gives an adverse event rate of 16.7% (95% CI 0-37.8%) with mild reactions.

By comparison, all 8 previously conducted OIT studies involved the use of roasted peanuts only and the corresponding adverse event rates were 45-95%, with frequent serious adverse events and withdrawal from OIT. The observed reduction in adverse events demonstrates the remarkable benefit of utilising boiled peanuts prior to roasted peanuts to achieve full desensitisation safely in the home and community outpatient environment. These are important observations with major implications for patients and the health system.

This pilot study provides the justification and support for an appropriately powered study to determine the safety and efficacy of home and community based OIT using boiled peanut. Based on the findings of the pilot study and that of our published paper, we have further modified the OIT regimen to incorporate the use of 12-hour boiled peanuts in phase 1, which will then be followed by 2-hour boiled peanuts in phase 2, and then roasted peanuts in phase 3. Our research demonstrates that 12-hour boiled peanuts have lower allergenicity than 2-hour boiled peanuts and raw peanuts, and an unchanged capability to stimulate T cell proliferation. We predict that commencing OIT with 12-hour boiled peanuts will provide sufficient desensitisation for commencement of 2-hour boiled peanuts with an even lower probability of reaction than demonstrated in our pilot study. We will also lower the roasted peanut starting dose to 1/16th to further reduce phase 3 reactions.

6. STUDY OBJECTIVES

6.1. Primary objective

To demonstrate the effectiveness and safety of a novel boiled-to-roasted peanut oral immunotherapy regimen in inducing desensitation in peanut allergic children.

7. STUDY DESIGN

7.1. Type of study

Single centre open label Phase 2 non-randomised clinical trial

7.2. Number of participants

The planned sample size is a total of 70 peanut allergic children

7.3. Expected duration

The study will be completed within a 2-year period. Each participant will undergo the peanut OIT protocol consisting of three phases over a 52-week period. Participants will be followed-up 6 months after completion of the OFC to assess continued consumption of peanuts.

7.4. Primary outcome measures

Proportion of children able complete peanut oral immunotherapy protocol and pass supervised exit oral food challenge with a cumulative dose of 3000 mg of peanut protein

without experiencing dose-limiting symptoms.

7.5. Secondary outcome measures

Prevalence and incidence of treatment-related adverse events, change in peanut skin prick test.

8. STUDY TREATMENTS

8.1. Peanut OIT protocol

1. Desensitisation is completed in three phases: 12-hour boiled peanuts for 12 weeks, then 2-hour boiled peanuts for 20 weeks, and finally roasted peanuts for 20 weeks, total 52 weeks (**Figure 1**).
2. Peanuts are eaten twice a day: morning (at breakfast) and evening (at dinner), except in the first week of each phase, when peanuts are eaten only once a day.
3. The starting dose of each stage is always equivalent to 1/16th raw peanut (whether 12-hour boiled, 2-hour boiled, or roasted).
4. The first 2 increments of each step are packaged in capsules, the contents of which are emptied onto a spoon of yoghurt. The remaining doses are eaten as fractions or intact peanuts and eaten at breakfast and their evening meal.
5. The doses are progressively increased every week and follow a simple pattern (**Table 1**).
6. Treatment adherence will be checked at each study visit and was required to be >90% (i.e. consumed 13 of 14 doses in the week prior to escalation to the next treatment Phase).
7. The initial dose at the beginning of each will be given in the outpatient clinic and participants were monitored until 2 hours after each dose.
8. In order to minimise treatment-related adverse events, participants will be advised to consume peanuts with food and avoid exercising within 2 hours of peanut ingestion.
9. Temporary withholding of peanuts are allowable in the instance of intercurrent illness or short vacations. If the duration of withholding peanuts exceeds 14 days, the participant must return to see the study investigator to review their dosing protocol and develop a revised treatment plan.
10. If symptoms occur at the time of dose-escalation, the dose will be reduced by 1 dose level (i.e. to the previous tolerated dose) and maintained at that reduced dose level for a 1-2 week period before attempting dose re-escalation.
11. Throughout the study, a 24-hour-per-day telephone hotline will be made available for immediate advice, especially for dosing instructions.

Figure 1. Boiled-to-roasted Peanut OIT Protocol

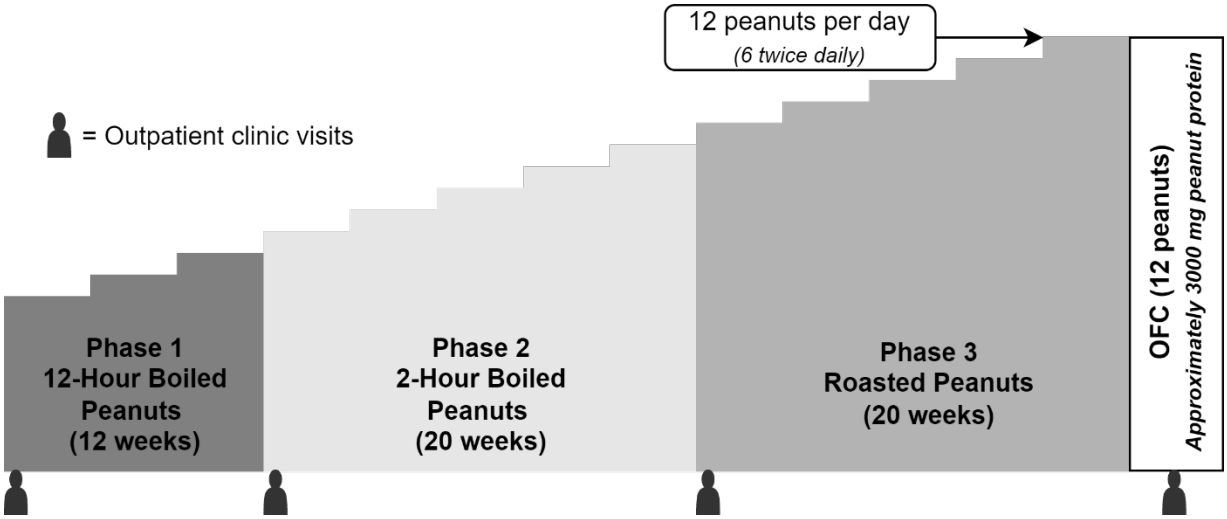

Table 1. Peanut OIT Protocol		
Week Number	**A.M. Dose**	**P.M. Dose**
Phase 1: 12-hour boiled peanut†		
1	None	1/16 peanut
2	1/16 peanut	1/16 peanut
3	1/16 peanut	1/8 peanut
4	1/8 peanut	1/8 peanut
5	1/8 peanut	1/4 peanut
6	1/4 peanut	1/4 peanut
7	1/4 peanut	1/2 peanut
8	1/2 peanut	1/2 peanut
9	1/2 peanut	1 peanut
10	1 peanut	1 peanut
11	1 peanut	2 peanuts
12	2 peanuts	2 peanuts
Phase 2: 2-hour boiled peanut†		
13	None	1/16 peanut
14	1/16 peanut	1/16 peanut
15	1/16 peanut	1/8 peanut
16	1/8 peanut	1/8 peanut
17	1/8 peanut	1/4 peanut
18	1/4 peanut	1/4 peanut

19	1/4 peanut	1/2 peanut
20	1/2 peanut	1/2 peanut
21	1/2 peanut	1 peanut
22	1 peanut	1 peanut
23	1 peanut	2 peanuts
24	2 peanuts	2 peanuts
25	2 peanuts	3 peanuts
26	3 peanuts	3 peanuts
27	3 peanuts	4 peanuts
28	4 peanuts	4 peanuts
29	4 peanuts	5 peanuts
30	5 peanuts	5 peanuts
31	5 peanuts	6 peanuts
32	6 peanuts	6 peanuts
Phase 3: Roasted peanut†		
33	None	1/16 peanut
34	1/16 peanut	1/16 peanut
35	1/16 peanut	1/8 peanut
36	1/8 peanut	1/8 peanut
37	1/8 peanut	1/4 peanut
38	1/4 peanut	1/4 peanut
39	1/4 peanut	1/2 peanut
40	1/2 peanut	1/2 peanut
41	1/2 peanut	1 peanut
42	1 peanut	1 peanut
43	1 peanut	2 peanuts
44	2 peanuts	2 peanuts
45	2 peanuts	3 peanuts
46	3 peanuts	3 peanuts
47	3 peanuts	4 peanuts
48	4 peanuts	4 peanuts
49	4 peanuts	5 peanuts
50	5 peanuts	5 peanuts
51	5 peanuts	6 peanuts
52	6 peanuts	6 peanuts
† Prior to boiling or roasting, raw jumbo peanuts weighed approximately 1000 mg		

8.2. Deviations to peanut OIT protocol

Doses can be omitted if participants become sick or go on vacation. If the omission period is longer than two weeks, changes to the schedule will be required through consultation with the Principal Investigator (Paediatric Allergist).

8.3. Exit oral food challenge

After reaching the end of the peanut OIT protocol, participants will undergo an oral food challenge which will consist of a cumulative dose of 12 roasted peanuts (12 g peanuts; approximately 3000 mg peanut protein). As a safety precaution, prior to undertaking the oral food challenge, participants and parents will be required to confirm ingestion of 12 roasted peanuts daily as part of the OIT protocol. The oral food challenge will involve participants consuming 1 whole peanut every 2-3 minutes until they have consumed a total of 12 peanuts. Those able to consume all 12 peanuts without experiencing dose-limiting symptoms will be considered to have achieved the primary efficacy end point.

8.4. Materials for oral immunotherapy

Boiled peanuts (using blanched, raw, jumbo-sized peanuts each weighing about 1 gram) will be produced by the Flinders Proteomic Facility with specially designed equipment. Thermal processing temperature will be kept to 98 +/- 1°C and monitored with a thermistor data logger. Each batch of processed peanuts (8 Kg per batch) will be compared to reference values for protein hydrolysis and reduced allergenicity by SDS PAGE, inhibition ELISA and western blot. To ensure dose accuracy at the start of oral immunotherapy (when the initial dosing is small), capsules containing ground boiled or roasted peanuts will be prepared by filling 000 gelatin capsules using a ProFiller 1100 Capsule Filling System. Roasted peanuts will be ground and then defatted by repeated extraction with acetone until the fat content is < 1%. All subsequent doses at ¼ peanut or higher will be simply cut from peanuts using a bread knife, or consumed as whole pieces of original peanuts. Raw and light roasted jumbo peanuts will be purchased from a large local nut company (Charlesworth Nuts, South Australia, Australia).

8.5. Packaging and labelling

Participants will be provided sufficient peanuts at the beginning of each treatment Phase. The clinical trial materials will be packaged and labelled in accordance with GMP including product ID, batch number, expiry date and include the statement “for clinical trial use only”.

8.6. Medication adherence

Participants will be provided with a diary to record each time peanuts are administered in accordance with the study protocol.

9. ENROLMENT AND RANDOMISATION

Potentially eligible participants will be identified based on referrals received by the Paediatric Allergist involved with the study. Eligible participants and their parents/carers will be provided information on the study by a research assistant or nurse. The information sheet will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. Study staff will conduct the informed consent discussion and will confirm that information provided is understood and answer any questions about the study. Written informed consent will then be obtained from participants and/or parents/carers prior to commencement of the study intervention.

9.1. Inclusion criteria

History: a clearly positive peanut-allergic history that includes ingestion of peanut, to be followed immediately by such reactions as rashes, angioedema, vomiting, abdominal pain, rhino-conjunctivitis, cough or wheeze. We will include patients who have received adrenaline treatment after the allergic reaction, but no more than a single dose.
Additional inclusion criteria include:
Age: 6 to 18
AND
Positive skin prick test (SPT) with wheal size ≥8mm
OR
Serum peanut-specific IgE (psIgE) >15 kU/L

9.2. Exclusion criteria

1. History of a severe anaphylactic reaction to peanut as defined by hypotension, collapse, loss of consciousness, hypoxia or ever needing three or more doses of intramuscular adrenaline or and intravenous infusion for management of an allergic reaction.
2. Significant medical co-morbidities such as severe asthma (either of: admission to hospital < 12 months ago; Multiple uses of Ventolin on a daily basis indicating poorly controlled asthma; Using more than 1 preventer; Had 3 courses of oral steroids in past 12 months), significant heart conditions (e.g. regular visits to a cardiologist), epilepsy (e.g. taking regular medications, requiring more investigations), and inflammatory bowel diseases
3. Concerns about psychosocial readiness of the child to participate in the study.
4. Contraindication to skin prick test e.g. diffuse dermatological conditions, severe dermatographism, or child unable to cease antihistamines.
5. The child or parents/guardians of the child object to have blood tests performed.
6. Children with parents/guardians who are unable to provide adequate supervision and adherence to the study protocol, or are unable to complete follow-up.

9.3. Treatment discontinuation

Study treatment will be permanently discontinued for any of the following reasons:

- Unacceptable side effects as determined by the participant or site investigator
- The investigator determined that continuation of treatment is not in the participant's best interest
- Failure to comply with the protocol, the participant declines further study treatment, or withdraws their consent to participate in the study.

9.4. Participant withdrawal

Participants or parents/carers are free to withdraw themselves and/or their children from the study at any time. The reasons for withdrawal will be recorded in the CRF and included in the final report.

Participants who discontinue treatment or are withdrawn from the study will not be replaced. Whenever possible, permission will be sought from participants who withdraw from the study to obtain as much data for the follow-up period as they will permit.

10. STUDY ASSESSMENTS AND PROCEDURES

Participants in the study will be asked to partake in a minimum of 5 study related visits to the outpatient clinic of the Site Investigator.

10.1. Study Assessments

In-person outpatient clinic study appointments:

Eligibility Assessment (Visit 0)

- Assess study eligibility (medical history, referral documentation)
- Conduct skin prick test
- Take bloods for peanut specific IgE

Study Visit 1

- Obtain informed consent (and assent)
- Discuss peanut OIT protocol
- Administer first dose of Phase 1 (12-hour boiled peanut) supervised in outpatient clinic

Study Visit 2

- Administer first dose of Phase 2 (12-hour boiled peanut) supervised in outpatient clinic

Study Visit 3

- Administer first dose of Phase 3 (12-hour boiled peanut) supervised in outpatient clinic

Study Visit 4

- Repeat skin prick test

- Take blood for peanut specific IgE
- Conduct open label oral food challenge

Telephone appointments:
Participants and their parents/carers will be phone each month to assess progress with peanut OIT protocol and collect information on adverse events.

Participants and their parents/carers will be contacted approximately 6 months following completion of their oral food challenge to assess whether they are continuing to consume peanuts.

11. ADVERSE EVENT REPORTING

Adverse events from commencement of treatment to exit oral food challenge will be monitored by patient diaries reviewed at study visits and through direct questioning by the study research assistant. Study visits occurred face-to-face at the beginning of each treatment phase, with monthly phone calls occurring between physical visits. Causality, severity, and association of adverse events with study treatment were assessed independently by two members of the research team. Any discrepancies in agreement will be reviewed by a third member of the research team.

Table 2: Causality definitions

Relationship	Description
Treatment unrelated adverse events	There is no or little evidence to suggest that there is a causal relationship (eg the event did not occur within a reasonable time after administration of the trial material). There is another reasonable explanation for the event (eg the participant's clinical condition or other concomitant treatment)
Treatment related adverse events	There is some or clear evidence to suggest a causal relationship (eg because the event occurs within a reasonable time after administration of the trial material), and the influence of other factors is unlikely or can be ruled out.

Anaphylaxis
Episodes of anaphylaxis will be defined according to the EAACI Taskforce position paper on Anaphylaxis in Children(Vetander 2011).

Anaphylaxis is likely when any one of the three following sets of criteria is fulfilled:

1. Acute onset of an illness (min to h) with involvement of:
   • Skin/mucosal tissue (e.g., generalized hives, itch or flush, swollen lips/tongue/uvula) AND
   • Airway compromise (e.g., dyspnea, stridor, wheeze/ bronchospasm, hypoxia, reduced PEF) AND/OR
   • Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to the allergen (min to h):
   • Skin/mucosal tissue (e.g., generalized hives, itch/flush, swollen lips/tongue/uvula)
   • Airway compromise (e.g., dyspnea, stridor wheeze/bronchospasm, hypoxia, reduced PEF)
   • Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
   • Persistent GI symptoms (e.g., nausea, vomiting, crampy abdominal pain)
3. Reduced BP after exposure to the allergen (min to h): low systolic BP (age-specific) or > 30% drop in systolic BP*

   * Low systolic BP for children is defined as < 70 mmHg from 1 month to 1 year; less than (70 mmHg + [2 x age]) from 1-10 years; and < 90 mmHg from age 11-17 years.

The severity of individual episodes of anaphylaxis will be graded according to the severity staging system below issued by the EAACI Taskforce position paper on Anaphylaxis in Children (**Table 3**). (Vetander 2011)

Table 3. Anaphylaxis severity grading	
Severity	**Definition**
1. Mild (skin & subcutaneous tissues, GI, &/or mild respiratory)	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. Moderate (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness and/or stridor; shortness of breath, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. Severe (hypoxia, hypotension, or neurological compromise)	Cyanosis or SpO2 ≤ 92% at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

Organ type and severity of treatment-related allergic events will be categorized according to a predefined standardized schedule adapted from the EAACI Taskforce position paper on Anaphylaxis in Children (**Table 4**).[9]

Table 4. Classification of treatment-related allergic events			
Organ	**Grade 1**	**Grade 2**	**Grade 3**

	(mild)	(moderate)	(severe)
Skin, rash (SR)	Localised urticaria, exanthema, wheal, pruritus	Generalised urticaria, exanthema, wheal, pruritus.	
Skin, angioedema (SA)	Swollen lip or eyelid	Swollen face	None
Gastrointestinal, upper (GIU)	Pruritus of throat or oral cavity.	Throat pain	None
Gastrointestinal, lower (GU)	Mild abdominal pain, nausea, emesis, diarrhoea	Moderate abdominal pain, recurrent emesis, recurrent diarrhoea	Severe abdominal cramps, continuous emesis, loss of bowel control
Respiratory upper (RU)	Nasal congestion, sneezing, rhinorrhoea.	None	None
Respiratory, lower (RL)	Intermittent cough	Repetitive cough, chest tightness, wheezing detectable via auscultation	Persistent or barking cough, audible wheeze without auscultation, dyspnoea, cyanosis, saturation <92%, swallowing or speaking difficulties, throat tightness, respiratory arrest
Cardiovascular (CV)	None	Pale face, mild hypotension, tachycardia (>15 beats/min above baseline)	Hypotension, dysrhythmia, severe bradycardia, cardiac arrest
Neurological (N)	Change in activity level, tiredness	"Light-headedness", feeling of "pending doom", somnolence, headache	Confusion, loss of consciousness, incontinence
Adapted from the following reference: Vetander M, Helander D, Lindquist C, Hedlin G, Alfvén T, Östblom E, Nilsson C, Lilja G, Wickman M. Classification of anaphylaxis and utility of the EAACI Taskforce position paper on anaphylaxis in children. Pediatric allergy and immunology. 2011;22(4):369-73.			

11.1. Safety reporting for RCT

Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial.

Table 4: Adverse Event Definitions

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)	Any AE or AR that at any dose: • results in death • is life threatening* • requires hospitalisation or prolongs existing hospitalisation** • results in persistent or significant disability or incapacity • or is another important medical condition***
* The term life threatening here refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that might hypothetically cause death if it was more severe (eg a silent myocardial infarction)	
** Hospitalisation is defined as an in-patient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisation for pre-existing conditions (including elective procedures that have not worsened) do not constitute an SAE *** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. Important AEs or ARs that may not be immediately life threatening or result in death or hospitalisation, but may seriously jeopardise the participant by requiring intervention to prevent one of the other outcomes listed in the table (eg a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not require hospitalisation, or development of drug dependency).	

Adverse events include:

- an exacerbation of a pre-existing illness
- an increase in the frequency or intensity of a pre-existing episodic event or condition
- a condition (regardless of whether PRESENT prior to the start of the trial) that is DETECTED after trial drug administration. (This does not include pre-existing conditions recorded as such at baseline – as they are not detected after trial drug administration.)
- continuous persistent disease or a symptom present at baseline that worsens following administration of the trial treatment

Adverse events do NOT include:

- Medical or surgical procedures: the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisation where no untoward or unintended response has occurred (eg elective cosmetic surgery)
- Overdose of medication without signs or symptoms

11.2. Serious Adverse Events (SAE)

The co-ordinating centre will provide the DSMC with reports of SAEs on an ongoing basis. Episodes of anaphylaxis should be reported within 24 hours of their occurrence (or upon the Investigator being notified of its occurrence) for forwarding to the DSMC if the event was associated with any of the following:

- Hospital Emergency Department Visit
- Hospitalisation
- More than 2 doses of epinephrine being used to treat the same episode
- Death

11.3. Emergency contact details

Dr Billy TAO – Paediatric Allergist
Allergy SA
Beulah Park SA 5067
billy.tao@sa.gov.au
Mobile: +61 418 802 380

11.4. HREC notification

The Investigator, or nominee, will also be responsible for reporting any serious adverse events to their Human Research Ethics Committee (HREC) as soon as possible and in any event within 72 hours. In agreeing to the provisions of the protocol, these responsibilities are accepted by the Investigator, or nominee.

If a participant dies, any post-mortem findings including histopathology, must be provided to the Coordinating Centre.

12. STATISTICAL METHODS

12.1. Sample size estimation

Based on our previous pilot study, 11 of 14 (78.6%) children were able to complete peanut oral immunotherapy and pass supervised exit oral food challenge (Unpublished). In order to estimate the true proportion of children able complete peanut oral immunotherapy and pass supervised exit oral food challenge within a margin of error of ±10%, based on an 80% expected proportion and 95% confidence interval, we require a minimum sample size of 70.

12.2. Statistical Methods- Outcomes

The study outcomes of effectiveness and safety will be reported using descriptive statistics. The primary outcome of the percentage of participants who can tolerate 12 roasted peanuts without allergic reaction at the completion of treatment will be reported.

Continuous outcomes are presented as mean (SD) (or median [IQR] with the range for skewed data) and categorical outcomes as percentages. The prevalence (n per child) and incidence rates (n per 1,000 doses) of adverse events will be reported.

Differences in continuous outcomes between groups will be compared using Student's t-test for normally distributed data and Kruskal Wallis test for non-normally distributed data. Differences in categorical outcomes between groups will be compared using Fisher exact test probability.
Changes from baseline in peanut prick test wheal size at the end of each treatment phase will be pairwise compared using Wilcoxon rank-sum test.

Statistical analyses will be undertaken using StateSE 14.

13. DATA MANAGEMENT

13.1. Data collection

Paper-based CRFs will be used for data collection, with data subsequently entered into a Microsoft Access Database.

13.2. Data storage

Paper based CRFs will be stored in a locked office at the study site. Only research staff directly involved in the study will have access to the information.

Electronic data will be stored in password protected files on the Flinders University server. Access to electronic data is granted only to research staff according to specific need.

13.3. Study record retention

Original/copies of study documents will be retained at the study site or in archives. Documents will be retained for at least 30 years after study completion in line with the data retention schedules for research involving minors. At the completion of this time documentation will be destroyed using confidential document disposal, by shredding with a commercial grade document shredder. The study electronic data will be stored indefinitely on Flinders University's secure servers with access only granted to authorised study personnel.

14. ADMINISTRATIVE ASPECTS

14.1. Regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice

(CPMP/ICH/135/95) annotated with TGA comments (Therapeutic Goods Administration DSEB July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007), and the NHMRC Australian Code for the Responsible Conduct of Research (©Australian Government 2007), and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008. To this end, no participant will be recruited to the study until all the necessary approvals have been obtained and the participant has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a subject. In this circumstance the Principal Investigator and HREC must be advised immediately.

14.2. Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators and research staff and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the coordinating centre. Coordinating Centre and regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records and pharmacy records for participants and their infants in this study subject to individuals having obtained approval/clearance through State/National Governments and HREC as required by local laws. The clinical study site will permit access to such records. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by HREC or regulatory agencies.

14.3. Independent HREC approval

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the HREC of each study site. A letter of protocol approval by HREC will be obtained prior to the commencement of the study, as well as approval for other study documents subject to HREC review.

14.4. Modifications of the protocol

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, study design, patient safety, or may affect a participants willingness to continue participation in the study is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All such amendments will be submitted to the HREC, for approval prior to becoming effective.

14.5. Protocol deviations

All protocol deviations must be recorded in the patient medical record and on the CRF and must be reported to the Principal Investigator. Protocol deviations will be assessed for significance by the Principal Investigator. Those deviations deemed to have a potential impact on the integrity of the study results, patient safety or the ethical acceptability of the trial will be reported to the HREC. Where deviations to the protocol identify issues for protocol review, the protocol will be amended.

14.6. Trial closure

The study may be terminated prematurely by the Principal Investigator or nominee if:

1. The number and/or severity of adverse events justify discontinuation of the study.
2. New data become available which raise concern about the safety of the study medications, so that continuation might cause unacceptable risks to subjects.

After such a decision, the Investigator must contact all participants within two weeks, and written notification must be sent to the Ethics Committee.

The Coordinating Centre may terminate the study at a study site/s at any time for any of the following reasons:

1. Failure to enroll participants
2. Major protocol violations
3. Inaccurate or incomplete data
4. Unsafe or unethical practices
5. Safe storage of the study products

In the event an Investigator terminates the study prematurely the Coordinating Centre requires the following:

1. Reasons for termination to be provided in writing.
2. All study supplies, including unused medications and CRFs be returned to the Coordinating Centre.
3. All 'Note for Guidance on Good Clinical Practice' (GCP) documents have been provided to the Coordinating Centre.
4. Investigative site must retain all study documents for at least 21 years after written notification to the Coordinating Centre.

15. USE OF DATA AND PUBLICATIONS POLICY

Publication of information and/or data related to this protocol in formats including, but not limited to, conference abstracts, posters or presentations; seminars, journal articles, public reports and internet postings, must be submitted to the HYPES Trial Management

Committee for consideration. Proposals for said activities must be within a reasonable time frame of any due dates. Approval for all said activities must have the written permission of the Chair of the Steering Committee or delegate prior to the event.

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HYPES: Peanut allergy desensitisation using sequential hypoallergenic and roasted peanuts

STUDY IDENTIFIER: HYPES

VERSION: 2

DATE: 4/05/2018

PRINCIPAL INVESTIGATOR:
Dr Tim Chataway

SPONSORING INSTITUTION:
Flinders Medical Centre
Flinders Drive, Bedford Park SA 5042, AUSTRALIA

Australian and New Zealand Clinical Trials Registry:
ACTRN12617000803392

Ethics Approval:
Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC EC00188)

PROTOCOL AMENDMENTS

Version	Revision Date	Amendments
2	4/05/2018	Addition of study investigator Dr Scott Morris (Section 1.1.) Inclusion of 6- to 8-week maintenance dosing phase prior to OFC (Section 8.3)

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007 – updated May 2015) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

I agree that the study will be conducted in accordance with the conditions outlined in the protocol (subject to any amendments). I have read and understood the protocol.

I understand that the information in this protocol is confidential. Publication of information related to this protocol in formats including, but not limited to, conference abstracts, posters or presentations; seminars, journal articles, public reports and internet postings, must be submitted to the Study Steering Committee for consideration. Proposals for said activities must be within a reasonable time frame of any due dates. Approval for all said activities must have the written permission of the Chair of the Steering Committee or their delegate prior to the event.

Investigator's Name:
Dr Billy Tao

Investigator's Signature:

Date: 4/05/2018

Study Site: Allergy SA

COORDINATING CENTRE:

Chair Steering Committee:
Dr Tim CHATAWAY
Flinders University

Chair Steering Committee signature:

Date: 4/05/2018

GLOSSARY OF ABBREVIATIONS

AI	Associate Investigator
AE	Adverse Event
CI	Chief Investigator
CRF	Case Report Form
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HREC	Human Research Ethics Committee
NHMRC	National Health and Medical Research Council
OIT	Oral Immunotherapy
SAE	Serious Adverse Event
SPT	Skin Prick Test
TGA	Therapeutic Goods Association

Contents

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1.2. Study Locations

Allergy SA
Beulah Park SA 5067 AUSTRALIA

Flinders Medical Centre
Bedford Park SA 5042 AUSTRALIA

2. STUDY MANAGEMENT

Flinders University is the nominated sponsor for the trial.

The Principal Investigator at each study centre will be responsible for the conduct of the study at their centre including informed consent, recruitment, data collection and maintenance of study documentation. Handling of investigational products will be the responsibility of clinical trial staff.

The Coordinating Centre Steering Committee, chaired by Dr Tim Chataway, will provide direct day-to-day management for the trial.

The core Steering Committee will meet regularly (at least monthly).

3. SERIOUS ADVERSE EVENT AND TRIAL MONITORING COMMITTEES

3.1. Serious Adverse Event Committee

A Serious Adverse Event (SAE) Committee will review all participant SAEs to determine whether there is any likelihood that involvement in the trial could have contributed. Cause of death will be determined from autopsy results or other hospital summaries by relevant medical personnel. This committee will meet three-monthly (or as required).

3.2. Data and Safety Monitoring Committee

An independent Data and Safety Monitoring Committee (DSMC) will be set up to review the yearly progress of the trial and provide feedback to the Steering Committee. The DSMC will review general study progress (recruitment, compliance, loss to follow-up, adverse events). The DSMC will also provide advice regarding external issues that may impact on the study (for example changes in clinical practice). The DSMC will review all SAEs. This committee will meet six-monthly or as required.

4. FUNDING

This study is supported by funding received from the Channel 7 Children's Research Foundation, South Australia, Australia.

5. INTRODUCTION AND BACKGROUND

5.1. Introduction

Peanut allergy affects up to 3% of Australian children (Osborne et al., 2011) and the prevalence rate is rising (Mullins et al., 2009, Sicherer et al., 2010). Most affected children will end up as peanut-allergic adults because less than 20% outgrow their allergic status over a 10-year period (Byrne et al., 2010).

Currently there is no safe method for treatment of peanut allergy. Management is simply avoidance and an action plan, which includes the prescription of an adrenaline self-injecting device (Epipen®) for those at high risk. Such an approach is far from ideal, and does not improve the quality of life of affected children and their families (Avery et al., 2003, Primeau et al., 2000, Bollinger et al., 2006). Further, avoidance cannot be guaranteed and accidental ingestion may be dangerous or even fatal (Boyce et al., 2010).

Oral immunotherapy (OIT) is a progressive desensitisation method giving patients increasing doses of an allergen until a target is reached over time. It is important to note that desensitisation can only provide temporary protection. If regular ingestion is discontinued the original allergy frequently returns. Consequently, patients need to continue ingesting the allergen regularly and possibly indefinitely.

This kind of approach has been extensively studied in the treatment of cow's milk, egg and peanut allergies (Nwaru et al., 2014). For peanut allergy, OIT using roasted peanut products (Hofmann et al., 2009, Clark et al., 2009, Jones et al., 2009, Blumchen et al., 2010, Varshney et al., 2011, Anagnostou et al., 2011, Anagnostou et al., 2014, Tang et al., 2015) showed early promise but further progress was hampered by the occurrence of frequent treatment-related adverse events (45-93%) and high rates of withdrawal (up to 35%).

OIT in its current form is not considered sufficiently safe for routine clinical use (Thyagarajan et al., 2010). As an illustration, Blumchen et al 2010 recorded 185 adverse events in 23 subjects receiving OIT, with 9/23 (39%) withdrawing. This figure did not include "subjective complaints" including oral-pharyngeal itchiness or abdominal pains. A recent Australian study by Tang et al 2015 demonstrated the lowest incidence of adverse events to date, but at 45% is still unacceptably high.

All published studies to date have required hospital involvement because of the high risk of adverse events. Subjects would need at minimum hospital-based outpatient supervision for up-dosing administration, and in a large number of cases hospital inpatient admission for OIT initiation. Such reliance carries substantial cost implications, adds further stress to our already

strained hospital resources, and creates insufficient capacity to provide OIT to all patients who would significantly benefit from it. There is urgent need for research focusing on the safe and efficacious administration of OIT that can occur in a community setting, representing a paradigm shift in the management of peanut allergy.

5.2. Study Rationale

In 2001 Beyer et al (Beyer et al., 2001) observed that the prevalence of peanut allergy in China was lower than Western countries and hypothesised that this was because peanuts consumed there were either boiled or fried rather than roasted. They demonstrated that boiling peanuts for 20 minutes was able to reduce IgE reactivity when compared to roasted peanuts. More recent publications have confirmed the reduction of IgE-reactivity as a consequence of boiling (Mondoulet et al., 2005, Maleki et al., 2010, Cabanillas et al., 2012, Kim et al., 2013), but none has investigated the effect of boiling longer than 60 minutes.

Our new OIT treatment is an extension of ideas from research undertaken as part of a previously funded Channel 7 Children's Research Foundation Grant (Reference number 14885) and is based on **two postulates**. The **first** is that a hypoallergenic peanut will result in fewer adverse events when compared to raw/roasted peanuts. Hypoallergenic forms of foods have been trialled in desensitisation of milk and egg allergies (mainly as baked products) (Nowak-Wegrzyn et al., 2008, Lemon-Mule et al., 2008, Turner et al., 2013), but not yet in peanut allergy. We have experimentally determined the boiling time required to produce hypoallergenic peanuts by boiling peanuts for up to 12 hours and analysing them with western blot, inhibition ELISA, skin prick test, mass spectrometry and flow cytometry (Tao et al., 2016). This paper by Tao et al is the first to establish the science of desensitisation using boiled peanuts. We found:

1. Extended boiling progressively reduced peanut allergenicity through a combination of leaching of allergens into cooking water, fragmentation of allergens, and denaturation of conformational epitopes.

2. 2-hour boiling led to an 8-fold reduction in IgE-binding capacity of boiled peanuts while 12-hour boiling led to a 19-fold reduction. Mass spectrometry revealed an increasing number of unique allergen peptides with longer boiling times (42-fold more in 12-hour, 5-fold more in 2-hour boiled than raw peanuts), while raw, 2-hour and 12-hour boiled peanuts were equivalent in their ability to stimulate T cell activation and proliferation.

We concluded that boiling progressively reduces IgE-reactivity without affecting T cell reactivity (which may be a prerequisite for desensitisation), making boiled peanut a suitable candidate for oral immunotherapy.

The **second** postulate is that boiled peanuts alone may not be able to fully desensitise peanut-allergic patients as they don't contain a complete repertoire of allergen epitopes, so a second OIT phase using roasted peanuts is required. Introducing boiled peanut in the first phase of OIT is predicted to reduce adverse events because boiled peanuts are hypoallergenic. Completion of a first phase of OIT using boiled peanut is also predicted to reduce adverse events in phase 2 because patients are at least partially desensitised. Therefore, the combination of boiled and roasted peanuts should provide full desensitisation with reduced adverse events.

We followed these ideas with a pilot feasibility study testing whether this novel 2-step desensitisation strategy can be safely carried out in the community setting independent of hospital involvement. It also facilitated further improvements to the original protocol and formed the basis of this proposal for a larger study.

The original pilot study utilised 2-hour boiled peanuts for partial desensitisation in phase 1 (7 months), followed by roasted peanuts for full desensitisation in phase 2 (5 months). After Phase 2, all patients were challenged with 10 roasted peanuts to prove that they were indeed desensitised. All up-dosing steps were carried out at patients' homes, and subjects were only required to visit Allergy SA for outpatient review at the first dose of boiled peanut (phase 1), the first dose of roasted peanut (phase 2), and the final 10-roasted-peanut challenge test.

We have preliminary data on a total of 12 children who have undergone OIT, all with clear peanut-allergic histories, positive skin tests, and a failed oral food challenge. At completion, all 12 (100%) children were able to ingest 10 roasted peanuts in one bolus dose with no reaction, demonstrating that desensitisation has been achieved. We expect to publish these results later this year.

In phase 1 (boiled peanut) three children recorded mild adverse events, providing an adverse event rate of 25% (95%CI 0.5-49.5%). Adverse events all occurred at the start of phase, and all resolved when the starting dose was reduced from ¼ 2-hour boiled peanut to 1/16 and then gradually increased back to 1/4 over 2 weeks.

In phase 2, of the 12 subjects who ingested roasted peanuts progressively, only 2 reported a single adverse event: one with mild abdominal pain and a small vomit, and the other with a feeling of "oral puffiness" but no visible swelling, both occurring after ingesting ¼-peanut at start of phase. As OIT progressed they were able to tolerate with ease all dose increments carried out at home without any problem. This gives an adverse event rate of 16.7% (95% CI 0-37.8%) with mild reactions.

By comparison, all 8 previously conducted OIT studies involved the use of roasted peanuts only and the corresponding adverse event rates were 45-95%, with frequent serious adverse events and withdrawal from OIT. The observed reduction in adverse events demonstrates the remarkable benefit of utilising boiled peanuts prior to roasted peanuts to achieve full desensitisation safely in the home and community outpatient environment. These are important observations with major implications for patients and the health system.

This pilot study provides the justification and support for an appropriately powered study to determine the safety and efficacy of home and community based OIT using boiled peanut. Based on the findings of the pilot study and that of our published paper, we have further modified the OIT regimen to incorporate the use of 12-hour boiled peanuts in phase 1, which will then be followed by 2-hour boiled peanuts in phase 2, and then roasted peanuts in phase 3. Our research demonstrates that 12-hour boiled peanuts have lower allergenicity than 2-hour boiled peanuts and raw peanuts, and an unchanged capability to stimulate T cell proliferation. We predict that commencing OIT with 12-hour boiled peanuts will provide sufficient desensitisation for commencement of 2-hour boiled peanuts with an even lower probability of reaction than demonstrated in our pilot study. We will also lower the roasted peanut starting dose to 1/16th to further reduce phase 3 reactions.

6. STUDY OBJECTIVES

6.1. Primary objective

To demonstrate the effectiveness and safety of a novel boiled-to-roasted peanut oral immunotherapy regimen in inducing desensitation in peanut allergic children.

7. STUDY DESIGN

7.1. Type of study

Single centre open label Phase 2 non-randomised clinical trial

7.2. Number of participants

The planned sample size is a total of 70 peanut allergic children

7.3. Expected duration

The study will be completed within a 2-year period. Each participant will undergo the peanut OIT protocol consisting of three phases over a 52-week period. Participants will be followed-up 6 months after completion of the OFC to assess continued consumption of peanuts.

7.4. Primary outcome measures

Proportion of children able complete peanut oral immunotherapy protocol and pass supervised exit oral food challenge with a cumulative dose of 3000 mg of peanut protein

without experiencing dose-limiting symptoms.

7.5. Secondary outcome measures

Prevalence and incidence of treatment-related adverse events, change in peanut skin prick test.

8. STUDY TREATMENTS

8.1. Peanut OIT protocol

1. Desensitisation is completed in three phases: 12-hour boiled peanuts for 12 weeks, then 2-hour boiled peanuts for 20 weeks, and finally roasted peanuts for 20 weeks, total 52 weeks (**Figure 1**).
2. Peanuts are eaten twice a day: morning (at breakfast) and evening (at dinner), except in the first week of each phase, when peanuts are eaten only once a day.
3. The starting dose of each stage is always equivalent to $1/16^{th}$ weight of a raw peanut (whether 12-hour boiled, 2-hour boiled, or roasted).
4. The first 2 increments of each step are packaged in capsules, the contents of which are emptied onto a spoon of yoghurt. The remaining doses are eaten as fractions or intact peanuts and eaten at breakfast and their evening meal.
5. The doses are progressively increased every week and follow a simple pattern (**Table 1**).
6. Treatment adherence will be checked at each study visit and was required to be >90% (i.e. consumed 13 of 14 doses in the week prior to escalation to the next treatment Phase).
7. The initial dose at the beginning of each will be given in the outpatient clinic and participants were monitored until 2 hours after each dose.
8. In order to minimise treatment-related adverse events, participants will be advised to consume peanuts with food and avoid exercising within 2 hours of peanut ingestion.
9. Temporary withholding of peanuts are allowable in the instance of intercurrent illness or short vacations. If the duration of withholding peanuts exceeds 14 days, the participant must return to see the study investigator to review their dosing protocol and develop a revised treatment plan.
10. If symptoms occur at the time of dose-escalation, the dose will be reduced by 1 dose level (i.e. to the previous tolerated dose) and maintained at that reduced dose level for a 1-2 week period before attempting dose re-escalation.
11. Throughout the study, a 24-hour-per-day telephone hotline will be made available for immediate advice, especially for dosing instructions.

Figure 1. Boiled-to-roasted Peanut OIT Protocol

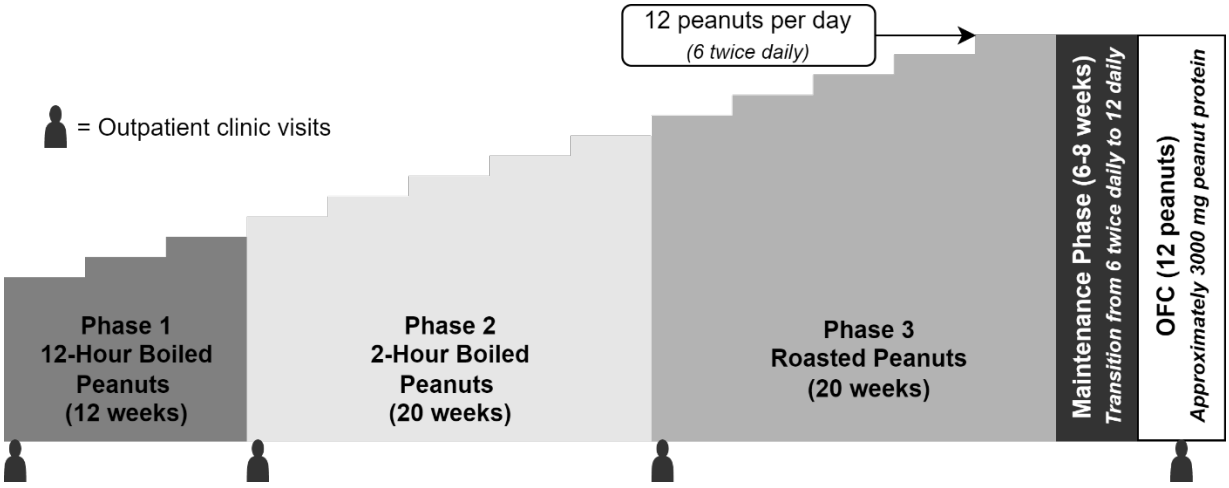

Table 1. Peanut OIT Protocol		
Week Number	**A.M. Dose**	**P.M. Dose**
Phase 1: 12-hour boiled peanut†		
1	None	63 mg ground peanut
2	63 mg ground peanut	63 mg ground peanut
3	63 mg ground peanut	126 mg ground peanut
4	126 mg ground peanut	126 mg ground peanut
5	126 mg ground peanut	1/4 peanut
6	1/4 peanut	1/4 peanut
7	1/4 peanut	1/2 peanut
8	1/2 peanut	1/2 peanut
9	1/2 peanut	1 peanut
10	1 peanut	1 peanut
11	1 peanut	2 peanuts
12	2 peanuts	2 peanuts
Phase 2: 2-hour boiled peanut†		
13	None	63 mg ground peanut
14	63 mg ground peanut	63 mg ground peanut
15	63 mg ground peanut	126 mg ground peanut
16	126 mg ground peanut	126 mg ground peanut
17	126 mg ground peanut	1/4 peanut
18	1/4 peanut	1/4 peanut
19	1/4 peanut	1/2 peanut
20	1/2 peanut	1/2 peanut
21	1/2 peanut	1 peanut
22	1 peanut	1 peanut

23	1 peanut	2 peanuts
24	2 peanuts	2 peanuts
25	2 peanuts	3 peanuts
26	3 peanuts	3 peanuts
27	3 peanuts	4 peanuts
28	4 peanuts	4 peanuts
29	4 peanuts	5 peanuts
30	5 peanuts	5 peanuts
31	5 peanuts	6 peanuts
32	6 peanuts	6 peanuts
Phase 3: Roasted peanut†		
33	None	31.5 mg ground defatted peanut
34	31.5 mg ground defatted peanut	31.5 mg ground defatted peanut
35	31.5 mg ground defatted peanut	63 mg ground defatted peanut
36	63 mg ground defatted peanut	63 mg ground defatted peanut
37	63 mg ground defatted peanut	1/4 peanut
38	1/4 peanut	1/4 peanut
39	1/4 peanut	1/2 peanut
40	1/2 peanut	1/2 peanut
41	1/2 peanut	1 peanut
42	1 peanut	1 peanut
43	1 peanut	2 peanuts
44	2 peanuts	2 peanuts
45	2 peanuts	3 peanuts
46	3 peanuts	3 peanuts
47	3 peanuts	4 peanuts
48	4 peanuts	4 peanuts
49	4 peanuts	5 peanuts
50	5 peanuts	5 peanuts
51	5 peanuts	6 peanuts
52	6 peanuts	6 peanuts
Maintenance Phase (Roasted peanut)†		
53-60	Transition from eating 6 peanuts twice daily to 12 peanuts once daily by increasing P.M. dose by 1 peanut each week.	
† Prior to boiling or roasting, raw jumbo peanuts weighed approximately 1000 mg		

8.2. Deviations to peanut OIT protocol

Doses can be omitted if participants become sick or go on vacation. If the omission period is longer than two weeks, changes to the schedule will be required through consultation with the Principal Investigator (Paediatric Allergist).

8.3. Maintenance dosing and exit oral food challenge

After reaching the end of the peanut OIT protocol, participants will enter a 6- to 8-week maintenance phase while awaiting an oral food challenge (OFC), where they transition to eating 12 roasted peanuts daily (from 6 peanuts twice daily). The oral food challenge will consist of a cumulative dose of 12 roasted peanuts (12 g peanuts; approximately 3000 mg peanut protein). As a safety precaution, prior to undertaking the oral food challenge, participants and parents will be required to confirm ingestion of 12 roasted peanuts daily as part of the OIT protocol. The oral food challenge will involve participants consuming 1 whole peanut every 2-3 minutes until they have consumed a total of 12 peanuts. Those able to consume all 12 peanuts without experiencing dose-limiting symptoms will be considered to have achieved the primary efficacy end point.

8.4. Materials for oral immunotherapy

Boiled peanuts (using blanched, raw, jumbo-sized peanuts each weighing about 1 gram) will be produced by the Flinders Proteomic Facility with specially designed equipment. Thermal processing temperature will be kept to 98 +/- 1°C and monitored with a thermistor data logger. Each batch of processed peanuts (8 Kg per batch) will be compared to reference values for protein hydrolysis and reduced allergenicity by SDS PAGE, inhibition ELISA and western blot. To ensure dose accuracy at the start of oral immunotherapy (when the initial dosing is small), capsules containing ground boiled or roasted peanuts will be prepared by filling 000 gelatin capsules using a ProFiller 1100 Capsule Filling System. Roasted peanuts will be ground and then defatted by repeated extraction with acetone until the fat content is < 1%. All subsequent doses at ¼ peanut or higher will be simply cut from peanuts using a bread knife, or consumed as whole pieces of original peanuts. Raw and light roasted jumbo peanuts will be purchased from a large local nut company (Charlesworth Nuts, South Australia, Australia).

8.5. Packaging and labelling

Participants will be provided sufficient peanuts at the beginning of each treatment Phase. The clinical trial materials will be packaged and labelled in accordance with GMP including product ID, batch number, expiry date and include the statement “for clinical trial use only”.

8.6. Medication adherence

Participants will be provided with a diary to record each time peanuts are administered in accordance with the study protocol.

9. ENROLMENT AND RANDOMISATION

Potentially eligible participants will be identified based on referrals received by the Paediatric Allergist involved with the study. Eligible participants and their parents/carers will be provided information on the study by a research assistant or nurse. The information sheet will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. Study staff will conduct the informed consent discussion and will confirm that information provided is understood and answer any questions about the study. Written informed consent will then be obtained from participants and/or parents/carers prior to commencement of the study intervention.

9.1. Inclusion criteria

History: a clearly positive peanut-allergic history that includes ingestion of peanut, to be followed immediately by such reactions as rashes, angioedema, vomiting, abdominal pain, rhino-conjunctivitis, cough or wheeze. We will include patients who have received adrenaline treatment after the allergic reaction, but no more than a single dose.
Additional inclusion criteria include:
Age: 6 to 18
AND
Positive skin prick test (SPT) with wheal size ≥8mm
OR
Serum peanut-specific IgE (psIgE) >15 kU/L

9.2. Exclusion criteria

1. History of a severe anaphylactic reaction to peanut as defined by hypotension, collapse, loss of consciousness, hypoxia or ever needing three or more doses of intramuscular adrenaline or and intravenous infusion for management of an allergic reaction.
2. Significant medical co-morbidities such as severe asthma (either of: admission to hospital < 12 months ago; Multiple uses of Ventolin on a daily basis indicating poorly controlled asthma; Using more than 1 preventer; Had 3 courses of oral steroids in past 12 months), significant heart conditions (e.g. regular visits to a cardiologist), epilepsy (e.g. taking regular medications, requiring more investigations), and inflammatory bowel diseases
3. Concerns about psychosocial readiness of the child to participate in the study.
4. Contraindication to skin prick test e.g. diffuse dermatological conditions, severe dermatographism, or child unable to cease antihistamines.
5. The child or parents/guardians of the child object to have blood tests performed.
6. Children with parents/guardians who are unable to provide adequate supervision and adherence to the study protocol, or are unable to complete follow-up.

9.3. Treatment discontinuation

Study treatment will be permanently discontinued for any of the following reasons:

- Unacceptable side effects as determined by the participant or site investigator
- The investigator determined that continuation of treatment is not in the participant's best interest
- Failure to comply with the protocol, the participant declines further study treatment, or withdraws their consent to participate in the study.

9.4. Participant withdrawal

Participants or parents/carers are free to withdraw themselves and/or their children from the study at any time. The reasons for withdrawal will be recorded in the CRF and included in the final report.
Participants who discontinue treatment or are withdrawn from the study will not be replaced. Whenever possible, permission will be sought from participants who withdraw from the study to obtain as much data for the follow-up period as they will permit.

10. STUDY ASSESSMENTS AND PROCEDURES

Participants in the study will be asked to partake in a minimum of 5 study related visits to the outpatient clinic of the Site Investigator.

10.1. Study Assessments

In-person outpatient clinic study appointments:

Eligibility Assessment (Visit 0)

- Assess study eligibility (medical history, referral documentation)
- Conduct skin prick test
- Take bloods for peanut specific IgE

Study Visit 1

- Obtain informed consent (and assent)
- Discuss peanut OIT protocol
- Administer first dose of Phase 1 (12-hour boiled peanut) supervised in outpatient clinic

Study Visit 2

- Administer first dose of Phase 2 (12-hour boiled peanut) supervised in outpatient clinic

Study Visit 3

- Administer first dose of Phase 3 (12-hour boiled peanut) supervised in outpatient clinic

Study Visit 4

- Repeat skin prick test
- Take blood for peanut specific IgE
- Conduct open label oral food challenge

Telephone appointments:

Participants and their parents/carers will be phone each month to assess progress with peanut OIT protocol and collect information on adverse events.

Participants and their parents/carers will be contacted approximately 6 months following completion of their oral food challenge to assess whether they are continuing to consume peanuts.

11. ADVERSE EVENT REPORTING

Adverse events from commencement of treatment to exit oral food challenge will be monitored by patient diaries reviewed at study visits and through direct questioning by the study research assistant. Study visits occurred face-to-face at the beginning of each treatment phase, with monthly phone calls occurring between physical visits. Causality, severity, and association of adverse events with study treatment were assessed independently by two members of the research team. Any discrepancies in agreement will be reviewed by a third member of the research team.

Table 2: Causality definitions

Relationship	Description
Treatment unrelated adverse events	There is no or little evidence to suggest that there is a causal relationship (eg the event did not occur within a reasonable time after administration of the trial material). There is another reasonable explanation for the event (eg the participant's clinical condition or other concomitant treatment)
Treatment related adverse events	There is some or clear evidence to suggest a causal relationship (eg because the event occurs within a reasonable time after administration of the trial material), and the influence of other factors is unlikely or can be ruled out.

Anaphylaxis

Episodes of anaphylaxis will be defined according to the EAACI Taskforce position paper on Anaphylaxis in Children (Vetander 2011).

Anaphylaxis is likely when any one of the three following sets of criteria is fulfilled:

1. Acute onset of an illness (min to h) with involvement of:
   - Skin/mucosal tissue (e.g., generalized hives, itch or flush, swollen lips/tongue/uvula) AND
   - Airway compromise (e.g., dyspnea, stridor, wheeze/ bronchospasm, hypoxia, reduced PEF) AND/OR
   - Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to the allergen (min to h):
   - Skin/mucosal tissue (e.g., generalized hives, itch/flush, swollen lips/tongue/uvula)
   - Airway compromise (e.g., dyspnea, stridor wheeze/bronchospasm, hypoxia, reduced PEF)

- Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
- Persistent GI symptoms (e.g., nausea, vomiting, crampy abdominal pain)

3. Reduced BP after exposure to the allergen (min to h): low systolic BP (age-specific) or > 30% drop in systolic BP*

   * Low systolic BP for children is defined as < 70 mmHg from 1 month to 1 year; less than (70 mmHg + [2 x age]) from 1-10 years; and < 90 mmHg from age 11-17 years.

The severity of individual episodes of anaphylaxis will be graded according to the severity staging system below issued by the EAACI Taskforce position paper on Anaphylaxis in Children (**Table 3**) (Vetander 2011).

Table 3. Anaphylaxis severity grading	
Severity	**Definition**
1. Mild (skin & subcutaneous tissues, GI, &/or mild respiratory)	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. Moderate (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness and/or stridor; shortness of breath, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. Severe (hypoxia, hypotension, or neurological compromise)	Cyanosis or SpO2 ≤ 92% at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

Organ type and severity of treatment-related allergic events will be categorized according to a predefined standardized schedule adapted from the EAACI Taskforce position paper on Anaphylaxis in Children (**Table 4**).[9]

Table 4. Classification of treatment-related allergic events			
Organ	**Grade 1 (mild)**	**Grade 2 (moderate)**	**Grade 3 (severe)**
Skin, rash (SR)	Localised urticaria, exanthema, wheal, pruritus	Generalised urticaria, exanthema, wheal, pruritus.	
Skin, angioedema (SA)	Swollen lip or eyelid	Swollen face	None
Gastrointestinal, upper (GIU)	Pruritus of throat or oral cavity.	Throat pain	None
Gastrointestinal, lower (GU)	Mild abdominal pain, nausea, emesis, diarrhoea	Moderate abdominal pain, recurrent emesis, recurrent diarrhoea	Severe abdominal cramps, continuous emesis, loss of bowel control
Respiratory upper (RU)	Nasal congestion, sneezing, rhinorrhoea.	None	None
Respiratory, lower (RL)	Intermittent cough	Repetitive cough, chest tightness, wheezing detectable via auscultation	Persistent or barking cough, audible wheeze without auscultation, dyspnoea, cyanosis, saturation <92%, swallowing or speaking difficulties, throat tightness, respiratory arrest
Cardiovascular (CV)	None	Pale face, mild hypotension, tachycardia (>15 beats/min above baseline)	Hypotension, dysrhythmia, severe bradycardia, cardiac arrest
Neurological (N)	Change in activity level, tiredness	“Light-headedness”, feeling of “pending doom”, somnolence, headache	Confusion, loss of consciousness, incontinence
Adapted from the following reference: Vetander M, Helander D, Lindquist C, Hedlin G, Alfvén T, Östblom E, Nilsson C, Lilja G, Wickman M. Classification of anaphylaxis and utility of the EAACI Taskforce position paper on anaphylaxis in children. Pediatric allergy and immunology. 2011;22(4):369-73.			

11.1. Safety reporting for RCT

Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial.

Table 4: Adverse Event Definitions	
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)	Any AE or AR that at any dose: • results in death • is life threatening* • requires hospitalisation or prolongs existing hospitalisation** • results in persistent or significant disability or incapacity • or is another important medical condition***
* The term life threatening here refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that might hypothetically cause death if it was more severe (eg a silent myocardial infarction)	
** Hospitalisation is defined as an in-patient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisation for pre-existing conditions (including elective procedures that have not worsened) do not constitute an SAE *** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. Important AEs or ARs that may not be immediately life threatening or result in death or hospitalisation, but may seriously jeopardise the participant by requiring intervention to prevent one of the other outcomes listed in the table (eg a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not require hospitalisation, or development of drug dependency).	

Adverse events include:

- an exacerbation of a pre-existing illness
- an increase in the frequency or intensity of a pre-existing episodic event or condition
- a condition (regardless of whether PRESENT prior to the start of the trial) that is DETECTED after trial drug administration. (This does not include pre-existing conditions recorded as such at baseline – as they are not detected after trial drug administration.)
- continuous persistent disease or a symptom present at baseline that worsens following administration of the trial treatment

Adverse events do NOT include:

- Medical or surgical procedures: the condition that leads to the procedure is the adverse event

- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisation where no untoward or unintended response has occurred (eg elective cosmetic surgery)
- Overdose of medication without signs or symptoms

11.2. Serious Adverse Events (SAE)

The co-ordinating centre will provide the DSMC with reports of SAEs on an ongoing basis. Episodes of anaphylaxis should be reported within 24 hours of their occurrence (or upon the Investigator being notified of its occurrence) for forwarding to the DSMC if the event was associated with any of the following:

- Hospital Emergency Department Visit
- Hospitalisation
- More than 2 doses of epinephrine being used to treat the same episode
- Death

11.3. Emergency contact details

Dr Billy TAO – Paediatric Allergist
Allergy SA
Beulah Park SA 5067
billy.tao@sa.gov.au
Mobile: +61 418 802 380

11.4. HREC notification

The Investigator, or nominee, will also be responsible for reporting any serious adverse events to their Human Research Ethics Committee (HREC) as soon as possible and in any event within 72 hours. In agreeing to the provisions of the protocol, these responsibilities are accepted by the Investigator, or nominee.

If a participant dies, any post-mortem findings including histopathology, must be provided to the Coordinating Centre.

12. STATISTICAL METHODS

12.1. Sample size estimation

Based on our previous pilot study, 11 of 14 (78.6%) children were able to complete peanut oral immunotherapy and pass supervised exit oral food challenge (Unpublished). In order to estimate the true proportion of children able complete peanut oral immunotherapy and pass supervised exit oral food challenge within a margin of error of ±10%, based on an 80% expected proportion and 95% confidence interval, we require a minimum sample size of 70.

12.2. Statistical Methods- Outcomes

The study outcomes of effectiveness and safety will be reported using descriptive statistics. The primary outcome of the percentage of participants who can tolerate 12 roasted peanuts without allergic reaction at the completion of treatment will be reported.

Continuous outcomes are presented as mean (SD) (or median [IQR] with the range for skewed data) and categorical outcomes as percentages. The prevalence (n per child) and incidence rates (n per 1,000 doses) of adverse events will be reported.

Differences in continuous outcomes between groups will be compared using Student's t-test for normally distributed data and Kruskal Wallis test for non-normally distributed data. Differences in categorical outcomes between groups will be compared using Fisher exact test probability.
Changes from baseline in peanut prick test wheal size at the end of each treatment phase will be pairwise compared using Wilcoxon rank-sum test.

Statistical analyses will be undertaken using StateSE 14.

13. DATA MANAGEMENT

13.1. Data collection

Paper-based CRFs will be used for data collection, with data subsequently entered into a Microsoft Access Database.

13.2. Data storage

Paper based CRFs will be stored in a locked office at the study site. Only research staff directly involved in the study will have access to the information.

Electronic data will be stored in password protected files on the Flinders University server. Access to electronic data is granted only to research staff according to specific need.

13.3. Study record retention

Original/copies of study documents will be retained at the study site or in archives. Documents will be retained for at least 30 years after study completion in line with the data retention schedules for research involving minors. At the completion of this time documentation will be destroyed using confidential document disposal, by shredding with a commercial grade document shredder. The study electronic data will be stored indefinitely on Flinders University's secure servers with access only granted to authorised study personnel.

14. ADMINISTRATIVE ASPECTS

14.1. Regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments (Therapeutic Goods Administration DSEB July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007), and the NHMRC Australian Code for the Responsible Conduct of Research (©Australian Government 2007), and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008. To this end, no participant will be recruited to the study until all the necessary approvals have been obtained and the participant has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a subject. In this circumstance the Principal Investigator and HREC must be advised immediately.

14.2. Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators and research staff and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the coordinating centre. Coordinating Centre and regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records and pharmacy records for participants and their infants in this study subject to individuals having obtained approval/clearance through State/National Governments and HREC as required by local laws. The clinical study site will permit access to such records. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by HREC or regulatory agencies.

14.3. Independent HREC approval

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the HREC of each study site. A letter of protocol approval by HREC will be obtained prior to the commencement of the study, as well as approval for other study documents subject to HREC review.

14.4. Modifications of the protocol

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent,

study design, patient safety, or may affect a participants willingness to continue participation in the study is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All such amendments will be submitted to the HREC, for approval prior to becoming effective.

14.5. Protocol deviations

All protocol deviations must be recorded in the patient medical record and on the CRF and must be reported to the Principal Investigator. Protocol deviations will be assessed for significance by the Principal Investigator. Those deviations deemed to have a potential impact on the integrity of the study results, patient safety or the ethical acceptability of the trial will be reported to the HREC. Where deviations to the protocol identify issues for protocol review, the protocol will be amended.

14.6. Trial closure

The study may be terminated prematurely by the Principal Investigator or nominee if:

1. The number and/or severity of adverse events justify discontinuation of the study.
2. New data become available which raise concern about the safety of the study medications, so that continuation might cause unacceptable risks to subjects.

After such a decision, the Investigator must contact all participants within two weeks, and written notification must be sent to the Ethics Committee.

The Coordinating Centre may terminate the study at a study site/s at any time for any of the following reasons:

1. Failure to enroll participants
2. Major protocol violations
3. Inaccurate or incomplete data
4. Unsafe or unethical practices
5. Safe storage of the study products

In the event an Investigator terminates the study prematurely the Coordinating Centre requires the following:

1. Reasons for termination to be provided in writing.
2. All study supplies, including unused medications and CRFs be returned to the Coordinating Centre.
3. All 'Note for Guidance on Good Clinical Practice' (GCP) documents have been provided to the Coordinating Centre.
4. Investigative site must retain all study documents for at least 21 years after written notification to the Coordinating Centre.

15. USE OF DATA AND PUBLICATIONS POLICY

Publication of information and/or data related to this protocol in formats including, but not limited to, conference abstracts, posters or presentations; seminars, journal articles, public reports and internet postings, must be submitted to the HYPES Trial Management Committee for consideration. Proposals for said activities must be within a reasonable time frame of any due dates. Approval for all said activities must have the written permission of the Chair of the Steering Committee or delegate prior to the event.

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