Protocol

3.3. Rationale for current study

The aim is to develop a new treatment for a common, serious and currently untreatable condition. We propose a definitive study of the efficacy of peanut oral immunotherapy (OIT) as a treatment for peanut allergy. Immunological mechanisms will be studied. We have conducted a pilot which demonstrated proof of concept; the present study includes larger numbers and a control group, with power to detect outcome at the 0.05 significance level. There will be two inter-dependent work packages (figure 1). Package 1 will be a randomized comparison of intervention versus the current best management. Package 2 will confirm efficacy in the waiting list group when subsequently treated and allow an estimate of the overall success rate to within 10%.

3.3.1 Recruitment

This is a single centre study. The intervention is complex and requires intensive clinical input; therefore a single centre with concentrated expertise makes more efficient use of resources than a multi-centre design. We can be confident of recruiting families from a broad geographical region. Following the recent national media publicity resulting from our pilot study[9], we received many enquiries from families across the UK, with peanut allergic children (the prevalence of peanut allergy is 2% in childhood). We are aware that this may result in participation bias with more motivated families with a vested interest in enrolling. The concern is that we recruit a sample of highly motivated individuals who show excellent compliance with treatment, which may not be representative of the response we might see from a more general sample recruited in our own clinic. We will therefore widen recruitment and include patients directly from our allergy clinic (we regularly review over 1500 peanut/nut allergic children in our clinic). We will perform a pre-planned sub group comparison of compliance and outcome between the national and locally recruited groups.

3.3.2 Expected natural resolution and success of therapy based on pilot results

For the power calculation, we have estimated the proportion of participants in the control group whom we expect to have undergone spontaneous resolution of their peanut allergy after a five-month waiting list control period. The current best estimate of spontaneous resolution is that it occurs in up to 20%, although this is in children with mild allergy, and occurs over a longer period. On current data, in the patients we are recruiting one would expect a resolution rate considerably below 20%. We have taken a conservative estimate of a 30% rate of natural resolution. For the success rate in the actively treated group we have used a conservative estimate of 64%. In our pilot study, 10/11 (91%) children who have completed peanut immunotherapy became completely tolerant to peanut and one tolerated 10 rather than 12 peanuts. The study has 90% power to detect a difference of 64% v 30% at the 0.05 significance level (see para10 for full details).

3.3.3 Peanut allergy diagnosis

It is vital to the study that the diagnosis is confirmed at the outset before group allocation, as determination of trial success depends on robust case definition. Therefore we will use the research gold-standard for diagnosis of active peanut allergy (DBPCFC) in all participants. The challenge is performed according to international consensus guidelines[12]. This challenge will also identify the threshold for reactivity (minimum amount of peanut required to cause a reaction) that will be used during minimization for group allocation. At the outset we expect all participants to have active peanut allergy, therefore for safety reasons the challenge commences at a low dose (1-100mg). Only participants with a positive DBPCFC will be included in the study.

3.3.4 Group allocation

Within an individual various factors including severity of allergy (from history and challenge), age, presence of asthma, and other active allergies, are known to influence reactivity and thus may influence the successful response to immunotherapy. Random group allocation may result in an imbalance of these factors between the active and control groups, leading to bias. To account for this, subjects will be allocated using minimization, with a random element using a weighting probability of 0.8.

3.3.5 Active intervention and control group

Peanut oral immunotherapy

Participants randomized to the active intervention arm will receive daily doses of peanut protein with two-weekly increments (see section 7). Participants will undergo six-seven months of immunotherapy before a second peanut challenge. Dose increments were well tolerated in our pilot study (achieved in 16/16) with no serious adverse events.

Control group

We have added a control group. There are no data on resolution in severe peanut allergy. The allergy may resolve in up to 20% of mildly peanut allergic children. This is thought to happen slowly over a number of years, rather than the relatively short period of our study. The control group will undergo peanut avoidance for five months (current practice) during which time they are on the 'waiting list' before receiving active intervention. A recent study demonstrates precedent of using a waiting list control group: 45 children with egg and cow's milk allergy were randomised to waiting list control or oral immunotherapy. After 18-24 months all control group subjects had a positive food challenge, demonstrating persistence of their allergy[7]. Subjects randomized to the control group will receive the current best management (peanut avoidance management plan) for six-seven months [in pilot duration was 58-210d (median 143d, mean 140d; about 5 months) n=12;]. This is provided using verbal and written information provision and training in the use of emergency medication and provision of an emergency treatment plan[11]. In an ideal world, we would administer a placebo to participants in our control arm and this was considered in great detail during development work. The main reason for not including a placebo is the impossibility of being able to adequately blind an active or placebo snack. Importantly, in our open design pilot study 70% of children had reactions immediately after taking an active dose, meaning participating families (and the investigators) would quickly work out which arm of the study they were on. After development work, we also concluded that it was not possible to adequately mask larger doses of peanut protein in a snack size acceptable for a child to eat every day (800mg is the equivalent of 5-7 peanuts). An alternative would be to provide a peanut-flavored placebo snack, but it would be unethical to leave families on either arm in doubt as to whether oral tolerance had been induced or not. Families who falsely assume they are on the active treatment arm may relax their allergen avoidance practice, thereby putting their child at increased risk of a reaction. We feel that a placebo arm would also hamper recruitment and compliance. The obvious disadvantage of not including a placebo snack is that subjects will know which study arm they are assigned to. This is offset by the fact that the primary outcome (peanut allergy) will be diagnosed on the basis of an objective measure: double blind, placebo-controlled food challenge (DBPCFC), the gold-standard for food allergy diagnosis in research. On peanut challenge, neither the investigator, the nurse, nor the participant will know whether the participant's dose is peanut or placebo, so it will be impossible for the outcome of the challenge to be influenced by that knowledge.

After completion of the second DBPCFC we will offer active immunotherapy to those in the waiting list control group who still have a diagnosis of peanut allergy. This is intended to maintain the high ethical standard of the study and encourage robust recruitment and compliance. We do not feel it is acceptable to families to wait for six months and attend for two peanut challenges, with their attendant risks, without a chance of receiving an active therapy. This will also to combine data from two active intervention groups to improve statistical power.

3.3.6 Severity

The Board has asked us to consider whether it would be appropriate to perform this study on a lower risk group. Arguably, those with the most to gain from this treatment are those at greatest risk of a severe reaction in the community. These include older children, those with low thresholds or a history of severe reactions, and those with asthma. We believe for the intervention in this study to be translated into a useful clinical treatment, studies must include these high-risk groups. Our pilot study included subjects with allergies of all severity represented. We demonstrated that it is possible to safely induce tolerance in subjects who have previously suffered an anaphylactic peanut reaction, those with very low documented thresholds for reactivity and those with asthma, so we have not excluded these individuals from participating.

3.3.7 Future development

The current proposal will advise on efficacy, mechanism and safety, also showing persistence of effect in the short to medium term. We are committed to future study that will investigate the optimal duration of maintenance treatment for long-term tolerance. In other forms of immunotherapy a maintenance treatment period of several years is required to induce a 'permanent' cure. This study is likely to catalyze and boost healthcare development for related allergic diseases. Not only will it provide a clinical treatment for peanut allergy, addressing a potentially life-threatening allergy in up to 2% of the childhood population, but it will also stimulate development of immunotherapy for other potentially severe food allergies e.g. tree nuts, sesame, fish, lupin and severe egg and milk allergy. We are currently experiencing an expansion in new food allergies: kiwi, mustard, lupin, fruits etc. The current study will provide a model for studying desensitization for novel food allergies as they emerge. Further no expensive vaccine is required. We have designed this intervention using cheap materials so that the treatment can be replicated at minimal cost and made widely available.

3.3.8 Outcome measure

The main goal of this trial is to compare the proportion of participants with peanut allergy after immunotherapy versus peanut avoidance. Therefore the outcome measure must be robust and objective.
We will be applying double blind placebo controlled peanut challenges to subjects in both groups.

3.3.9 Immunological Assessments (mechanism)

The state of the art is that it is known that clinical tolerance during natural resolution or immunotherapy to inhaled allergens is accompanied by several immunological changes[13]. Results from small studies of different parameters for food allergy indicate early changes such as recruitment of regulatory T cells (producing IL-10) or production of specific IgG antibodies, mid term changes including reversal of the ratio of allergy-skewed T helper cells and late changes such as reduction in specific IgE. Changes in histamine receptor expression have recently been noted during bee venom immunotherapy. However, there has not been a single longitudinal or more comprehensive examination of these features in food allergy immunotherapy, in comparison with a control group. A key difficulty in providing immunotherapy is knowing when a permanent cure has been effected and active treatment can be stopped. Current clinical biomarkers e.g. serum specific IgE or skin prick tests remain positive long after successful inhalant immunotherapy. As a result the duration of immunotherapy is probably overestimated. Definition of a surrogate marker would be of great clinical value in individualising treatment. A further important clinical question is defining in which group of patients immunotherapy is likely to be tolerated and successful, identifying such a biomarker will streamline provision of care in the future.
This clinical trial of oral tolerance induction will be accompanied by a series of studies that will permit us to identify the following:

- Longitudinal characterisation of early, mid and late-term molecular mechanisms associated with the development of oral tolerance during peanut immunotherapy
- Biomarkers that may identify the achievement of tolerance to peanut in individuals

- Indicators of an active (immunomodulatory) response to oral tolerance induction during successful therapy.
- Biomarkers to help predict a positive outcome for oral tolerance induction

Our strategy for identifying these biomarkers and indicators will involve longitudinal comparisons of immune-response profiles over time within individuals, as tolerance is achieved (or when it is unsuccessful). We will also compare immune-response profiles between individuals on the control arm and those on the active intervention arms. Qualitative, quantitative, and time-dependent changes in allergen immune-response profiles of participants who do or do not develop tolerance to peanut will provide data for testing current hypotheses and generating new hypotheses to explain underlying tolerogenesis mechanisms. To identify the biomarkers associated with underlying tolerance mechanisms in operation at the time of sampling, we will perform analysis of the immune-response profiles at enrolment and at 14 days (early-point), 75 days (mid-point) and 150 days (late-point) after active intervention or avoidance. Integration of biological responses with clinical outcomes will provide a means to identify the potential biomarkers outlined above. Assays at these time points for all subjects will include:
•Total and crude peanut-specific IgE, peanut-specific IgG and IgG4
•Ara h 1-3, 8 and 9-specific IgE, IgG and IgG4 will be measured
Peanut specific T helper cell proliferation and cytokine response (supernatant IL-4/IFN gamma and IL-10 by ELISA; intracellular IL-4, IFN gamma and IL-10 by flow cytometry)
In a subset of older subjects able to provide a larger blood volume (200mls; >14yrs) we plan :

- Isolation of basophils and assay of activation markers CD63 and CD203c measured by flow cytometry during peanut allergen stimulation.
- Ratio of histamine HR1 to HR2 receptor mRNA on basophils by semi-quantitative polymerase chain reaction

Specimens may also be used in future immune response or biomarker assays to re-evaluate biological response as research tests are developed.

4. Research objectives:
The overarching objective is to determine efficacy of oral immunotherapy in peanut allergy. We will determine whether the planned intervention is successful in the intervention group compared to control (package 1),and whether it is successful when offered to the control group (package 2). Other objectives include identification of immunological changes over time, and improvement in quality of life scores.

5. Research design
5.1 Design. This is a randomized controlled trial of a novel active intervention (peanut oral immunotherapy) versus the status quo (peanut avoidance) in 104 participants (7-18yrs) with peanut allergy (figure 1). One hundred and four participants will be recruited and undergo characterization by history, allergy testing, blinded peanut challenge and quality of life questionnaire. Peanut allergy will be confirmed in all participants by DBPCFC. Subjects are then allocated to either active or control group. Central minimization with weighting will be used to account for variation in severity of reaction (from history and challenge), peanut threshold dose (from challenge), age, sex, asthma, and allergies to other foods. After six-seven months subjects will undergo a DBPCFC (primary outcome measure). Subjects in the control group will receive current best management (a comprehensive management plan focusing on peanut avoidance) for six-seven months followed by DBPCFC. Those in the control group who still have a diagnosis of peanut allergy will be invited to undertake peanut oral immunotherapy followed by a final peanut challenge.

5.2 Ongoing review

The Data Monitoring Committee (DMC) will review safety data on an ongoing basis and report to the TSC. The DMC may stop enrolment or participation in the trial at any moment if it concludes that there are significant safety concerns.

5.3 Stopping enrolment

Enrolment in the trial will be stopped pending review if any of the following occur

- Any death
- A participant is admitted to the intensive care unit for a study-related adverse event
- Any participant in the active treatment group experiences life-threatening anaphylaxis

5.4 Premature termination of trial interventions

Trial intervention will be prematurely terminated for a participant if, in the judgment of the investigator, further participation in the trial would be deleterious to the participant's health Participants will be prematurely terminated from the trial for either of the following: withdrawal of consent, or failure to return. Such participants will not be replaced.

6. Study population:

6.1 Inclusion criteria

1. Subjects aged between 7 and 15 years of age
2. Subjects with peanut allergy confirmed by a clinical history of a typical rapid onset immediate type hypersensitivity reaction to definite peanut ingestion.
3. Positive skin prick test to peanut (extract ALK-Abello, Hørsholm, Denmark) defined by weal ≥3mm in the presence of a negative control and positive histamine control.
4. Positive double blind placebo controlled food challenge performed according to international consensus guidelines[12]
5. Informed consent obtained from parent / guardian or participant, as appropriate.

6.2 Exclusion Criteria

1. Clinically significant chronic illness, except for eczema, rhinitis or asthma.
2. Suspected or diagnosed allergy to peanut protein in care provider or current household member.
3. Unwillingness or inability to comply with study requirements and procedures.

7. Planned interventions: (see figure 1) All subjects will undergo initial clinical characterization by history and allergy testing (skin prick tests), and DBPCFC with peanut at study enrollment. DBPCFC will confirm clinical peanut allergy and identify the amount of peanut required to cause a reaction (threshold level).

7.1 Peanut oral immunotherapy

Subjects randomized to the active intervention arm will receive daily doses of peanut protein starting at 1mg per day. This is given as peanut flour (50% protein) and is mixed into a carrier which is known to be tolerated (e.g. yoghurt). The first dose is administered on the Clinical Research Ward (CRW) followed by a 2 hour observation period. Subsequently this dose is taken daily at home by participants. Every 2 weeks subjects return to the CRW for a dose increase and 1 hour of observation. The dose increments are 1, 5, 12, 25, 50, 100, 200, 400 and 800mg. The final dose (or if this is not tolerated, the highest tolerated dose) is taken for at least six weeks until six-seven months post randomization when a DBPCFC is performed.

Provide each participating family with:

- Symptom advice sheet (containing advice to avoid strenuous exercise for 2 hours after home dose)
- Contact information (explain 24 hour contact system)
- Non-sedating oral antihistamine and injectable adrenaline device
- Training on adrenaline autoinjector use (provide trainer pen)

- Emergency treatment plan
- Subjects are asked to complete a symptom diary, noting type and duration of symptoms and any exacerbating factors (e.g. exercise, excessive tiredness, systemic illness).

If symptoms are experienced during updosing on the Clinical Research Ward then do the following:

- If wheeze / breathlessness / reduced PEFR / vomiting occur then provide the next lowest dose for daily active intervention. It should be noted that this action was not required in the pilot study. If the dose causing the reaction was the starting dose (1mg), then provide 0.5mg peanut protein for daily active intervention. Contact daily to reassess.
- If mild abdominal pain, oral itching or urticaria occurs then provide reassurance and continue the current dose. Contact daily to reassess
- If other symptoms occur that are intolerable to the subject that the dose can also be reduced at the investigator's discretion.

If any symptoms are experienced by participants at home, families are advised to contact the study team. Serious adverse events will be handled separately as below in section 9.

Make the following assessments and provide advice as indicated:

- If wheeze / breathlessness / vomiting occurs within 2 hours of a single dose taken at home then reduce the dose to the next lowest, and contact daily to reassess
- If abdominal pain / rhinitis / oral itching occurs within 2 hours of a single dose taken at home then continue with the current dose and contact daily to reassess
- If episodes of abdominal pain / rhinitis / oral itching occur every day for 10 days, then reduce to the next lowest daily and contact daily to reassess
- After a dose reduction, attempt to increase dose again as per protocol at the next scheduled visit to the Clinical Research Ward.

Intercurrent illness, if symptoms occur which are not temporally related to taking a dose (for example, but not limited to wheeze, rhinitis, vomiting, fever, diarrhoea or rash) occurring more that 2 hours after a dose then consider reducing dose and advise to seek medical consultation, contact daily to reassess. Subjects will have 24-hour access to the study team via a dedicated mobile telephone. There will also be a single email address checked daily Mon-Fri for non-urgent enquiries. The telephone duty will be rotated between Dr Clark, Dr Anagnostou and Sr King on an equal basis. Dr Anagnostou and Sr King will be able to contact Dr Clark or Dr Ewan for further advice if needed. This system was used during the pilot study and proved helpful to the participants, but not over burdensome to the research team.

Peanut avoidance advice (waiting list control)

Provide written and verbal peanut avoidance advice[11]. Advise complete peanut avoidance for five months until repeat oral challenge. Provide emergency medication as for active group and train in its use (together with emergency treatment plan)[11].

7.2 Compliance

During our pilot study there was a single drop out from 20 participants (16yr old; withdrew after first dose, no reason given). We noted that approximately 70% of subjects experienced mild transient effects of therapy (e.g. oral itching or mild abdominal pain) at least once during therapy, these were mostly experienced at the lower doses (e.g. 5mg), with tolerance to higher doses (>50mg) being the norm. Despite this the pilot study drop-out rate was extremely low. This is in part due to the high level of motivation amongst study families and also the expectation of a successful outcome at the end of therapy. This feeling was reinforced by the 'feedback' of improving tolerance to open peanut administration as doses are increased. We therefore do not expect a high rate of drop-outs in the current study. Participants will be carefully supported and according to the protocol above if there are signs of a dose causing persistent problems, then a lower dose will be administered. However, we have increased our proposed recruitment number by 10% to take account of this. The provision of active intervention to the control group after the waiting period is an important mechanism to ensure adequate recruitment and compliance in both arms of the study.

8. Proposed outcome measures:

8.1 Primary Endpoint (outcome) Primary analysis will be the comparison of the proportion of participants in active versus control groups who pass a peanut challenge test at the end of six months.

8.2 Secondary Endpoints

-
- The proportion of participants from the control group who pass a peanut challenge after receiving the active intervention
- The fold increase and absolute increase in threshold (amount of peanut protein tolerated) for the active and control group members after intervention.
- Quality of life scores in active treatment group after intervention and in control group after waiting list control
- Quality of life scores before and after intervention for both active and control groups (disease-specific validated tool
- Immunological outcomes: results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to peanut
- Safety outcomes; incidence and nature of adverse events
- Recruitment source: assessment of compliance and efficacy in groups recruited nationally and locally
- Compliance: assessments of acceptability and compliance during intervention

9. Assessment and follow up:

9.1 Assessment of efficacy/effectiveness: Determination of peanut allergy at various assessment points will be achieved by double blind placebo controlled peanut challenges

9.1.1 Challenge timing

All participants will undergo a DBPCFC at enrolment (we expect the majority to react during this) After six-seven months the active and control group subjects undergo a second DBPCFC. The control group will then undergo immunotherapy, after six-seven months they also undergo a final DBPCFC.

9.1.2 Perform clinical assessment

All subjects will be assessed to determine their suitability for a challenge. A participant's eligibility for a challenge is guided by the following criteria:

The subject has had no acute exacerbation of allergic signs or symptoms within the last week, has not received short-acting beta-2 agonists for 12 hours, long-acting beta-2 agonists for 24 hours, short-acting antihistamines in the last 48 hours, or long-acting antihistamines in the last 7 days. The subject has no concurrent illness. Prior to conducting challenges, do the following: Ensure that both oxygen and suction are in working order. Ensure that all steps of the anaphylaxis protocol are in place and that all emergency drugs are readily available. Record baseline observations. One of the following challenge methods will be chosen depending on group allocation and position in the trial (see flow chart figure 1)

9.1.3 DBPCFC

On a day prior to the challenge, the study nurse will prepare the challenge doses in active (low dose recipes: 5, 50, **and** 100mg, ~~200mg~~) and placebo forms. A single peanut concentration mixture is used in each recipe for safety (i.e. each dose in the challenge is physically larger than the previous one so dose order cannot be accidentally reversed). All steps, including peanut weighing and labelling as active or placebo are double-checked with a colleague, who must be present throughout preparation. The order of each challenge is to be randomized independently by the laboratory research associate (independent of the clinical study) using an online minimization tool (www.randomizer.at). Record the randomization key in the challenge file. Each dose is administered in order separated by a **10-60m** interval and subjects are observed for 2 hours after

the last dose. If subjective symptoms of an allergic reaction occur (see table 1, section 9.1.6) they will be recorded and the apparent eliciting dose will be noted. If no objective signs appear, then the next highest challenge dose will be given in order. If subjective symptoms occur again with the next dose or are severe, then terminate the challenge and treat as appropriate. If objective signs occur at any stage then the eliciting dose is noted and the challenge is terminated, without further dose progression. A negative DBPCFC is followed by an open challenge of up to 12 peanuts. The outcome of the challenge (positive or negative-see below) and the apparent eliciting threshold dose will be recorded in the participant record.

9.1.4 Determining challenge outcome

For DBPCFCs challenge arms will be stopped and recorded as positive if one objective or two subjective symptoms occur (see table 1).

Table 1
Classification of the signs encountered during food challenges
1. Subjective
Oral itching, nausea, mild abdominal pain with no change in behaviour
2. Objective
Urticaria (>1 site), angioedema (>1 site), wheeze, reduced peak expiratory flow rate >/=20%, stridor, dysphonia, acute rhinoconjunctivitis, severe abdominal pain with behaviour change (e.g. doubling up, clinginess), vomiting, hypotension for age (not vaso-vagal), elevated serum mast cell tryptase (retrospective).

9.2. Adverse events

Safety data will be recorded on a specifically designed case report form (CRF). All serious adverse events (SAEs) will be reported on an SAE report in addition to CRFs. Safety data will be reviewed periodically by the DMC. The DMC has the authority to withdraw any participant and/or terminate the trial because of safety findings.
Adverse events that are classified as serious must be reported promptly and appropriately to the NIHR, Cambridge University (sponsor), principal investigators in the trial, and the Ethics Committee. This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0 (December 12, 2003).

9.2.1 Adverse Event definitions

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation. An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first. A serious adverse event is defined as any adverse event that suggests a significant hazard. This includes but is not limited to any of the following:
1. Death: A death that occurs during the study must be reported whether considered treatment related or not.
2. A life-threatening event: A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be

considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Other conditions specified in the protocol.
An adverse event is considered as 'unexpected' when its nature or severity is not consistent with the investigator's protocol.

9.2.2 Collection and recording of adverse events
Adverse events will be collected from the time the participant provides consent until the time the event resolves or until 30 days after the participant completes study treatment. Adverse events may be discovered through observing and questioning the participant or receiving an unsolicited complaint and questioning the participant in an objective manner. Throughout the study, the investigator will record all adverse events on the appropriate adverse event CRF regardless of their severity or relation to study medication or study procedure. The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes. SAEs will be recorded on the adverse event CRF and health authorities notified.

9.3 Grading and attribution of adverse events
9.3.1 Grading Criteria
The study site will grade the severity of adverse events experienced by study participants according to the criteria set forth in the NCI-CTCAE Version 3.0. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual: Grade 1 = mild adverse event. Grade 2 = moderate adverse event. Grade 3 = severe and undesirable adverse event. Grade 4 = life-threatening or disabling adverse event. Grade 5 = death. All adverse events will be reported and graded whether they are or are not related to disease progression or treatment.

9.3.2 Attribution Definitions
The relation, or attribution, of an adverse event to study participation will be determined by the investigator and recorded on CRF and/or SAE reporting form. The relation of an adverse event to the study treatment will be determined using the descriptors and definitions provided in Table 1. For additional information consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

9.4 Reporting serious adverse events
9.4. 1 Timeline
Serious adverse events must be reported to the PI within 24 hours
9.4.2 Options for Reporting Serious Adverse Events All SAEs will be reported to the Cambridge LREC, DMC AND NIHR where in the opinion of the Chief Investigator the event was related (resulted from the administration of any of the research procedures) and unexpected (an event not listed in the protocol as an expected occurrence). Reports will be submitted within 15 days of the CI being made aware of the event.

10. Proposed sample size: The study will be carried out at a single centre (Wellcome Trust Clinical Research Facility at Addenbrookes Hospital, Cambridge). We will enroll 104 subjects, with an estimated 49 randomized to each group (including a 5% uplift to account for withdrawals).
Package 1 Aim: A randomized controlled comparison of intervention versus waiting list control
For the primary analysis a Fisher's exact test will have 90% power, using a 0.05 two-sided significance level, to detect a difference between group percentages passing the peanut challenge test of 64% vs 30% when the sample size per group is 49 (reference: nQuery 4.0, StatSol Ltd, Ireland). Allowing for just over 5% loss-to-follow-up rate this implies recruiting 104 participants overall to be allocated equally into the active intervention vs waiting list control groups.

Package 2 Aim: To confirm efficacy in the waiting list group when treated, alone and in combination with the active group to allow overall estimate of success rate to within 10%. It will be possible to conclude the true efficacy of the intervention would be within 10% of the overall sample-based point estimate when combining those who received the intervention with or without a waiting period, anticipated to be approximately n=35 and n=50, respectively, with 95% confidence

11. Statistical analysis:

11.1 Analysis samples

The following groups will form samples for analysis

11.1.1 Intention to treat: All subjects allocated to each arm will be analyzed together as representing that treatment arm, whether or not they have completed the prescribed regimen.

11.1.2 Per protocol analysis: The criteria for per protocol analysis of the outcome of peanut challenge at the end of immunotherapy will consist of: Tolerance and continuation of immunotherapy up to 800mg protein

11.2 Endpoint analysis

11.2.1 Analysis of primary endpoint: The main analysis will compare the proportion of subjects who pass a peanut challenge in the intervention and control arms six-seven months after randomization, using a Fisher's exact test at the $p<0.05$ level of significance.

11.2.2 Analysis of secondary endpoints. Quality of life scores will be compared before and after intervention by pair wise comparison within individuals, and group medians will be compared using methods for non-parametric data. The results of cellular and humoral assessments of immune responses will be compared using methods for non-parametric data (e.g. comparison of medians of serum peanut specific IgG4 at different time points by Mann-Whitney U test). The incidence of adverse events will be compared between the intervention and control groups using a two-tailed Fisher's exact test at the $p<0.05$ level of significance. For package 2, the proportion of patients who may expect success will be calculated, with 95% confidence intervals. The influence of severity, age, sex, other allergy on immunotherapy success will be assessed by multiple logistic regression analysis.

11.3 Frequency of analysis

There will be no formal interim analysis, but there will be a DMC for safety purposes (see section 13)

12. Ethical arrangements

We obtained ethical consent for our pilot study which had an identical design to the active intervention arm of this proposal. We have the additional consideration of the control group in this proposal. Subjects in the control group will undergo a peanut challenge before and after a six-seven month period of current best management. Therefore these subjects will not be disadvantaged by comparison with current best standards of treatment, we have the best published outcome for peanut allergy[11].

Subjects will be provided with an information sheet that will be approved by the Cambridge LREC, describing the potential benefits and risks of participation. We will then offer to meet the families for further discussion. Informed consent will be obtained from parents for children (<16y). Ethical approval will be considered by the Cambridgeshire 2 LREC on 25^{tht} September 2009.

13. Research Governance This study will be conducted using good clinical practice (GCP), as delineated in the MRC Guidelines on Good Clinical Practice in Clinical Trials. Before initiation, the protocol and the informed consent documents will be reviewed and approved by our local ethics committee. The study will be sponsored by University of Cambridge Clinical School (Dept Medicine). The study may be inspected by the University under their remit as sponsor to ensure adherence to Good Clinical Practice.

In accordance with the Research Governance Framework we will nominate a trial steering committee (TSC). An independent chair will be appointed and membership will include Dr Clark, Dr Ewan, Sr King and Dr Palmer (trial statistician), it will also include one independent clinician and David Reading, of the Anaphyalxis Campaign (founder and recent chief executive). The TSC has responsibility for strategy and direction and has the responsibility of ensuring the project's aims are delivered on time. The TSC will invite EME program members to attend and report directly to the EME. We will also convene a Data Monitoring Committee (DMC) which will comprise one statistician and two independent clinicians. The DMC will report to the TSC and also report any serious adverse events directly to the EME program

14. Project timetable and milestones: See table 2, appendix

15. Expertise

Dr Pamela Ewan is co-principal investigator and group leader. Dr Ewan has an outstanding academic record in all areas of allergy and is a leading international figure in allergy clinical practice and research. Dr Ewan has been at the forefront of developing allergy services locally and nationally and was recently appointed Commander of the British Empire as a result of her endeavors. Her previous experience in the development of and mechanistic investigation of venom immunotherapy puts her in a uniquely advantageous position to direct and oversee this project. She has published widely on food allergy and her research has led to improvements in clinical practice. Dr Andrew Clark is the clinical supervisor and co-principal investigator. His long standing commitment to projects investigating the underlying mechanisms and clinical features of food allergy in children will prove particularly valuable in the undertaking of this clinical trial. He has published widely on clinical features, diagnosis, management and the pathophysiology of food allergy. Dr Clark currently leads the pilot study. Dr Clark has run clinical trials examining the pathophysiology of food allergy in childhood, for the past seven years and has extensive experience in performing food challenges and immunotherapy under research conditions. Dr Clark is responsible for project management, ensuring objectives and milestones are achieved, clinical training of Dr Anagnostou and supervision of peanut challenges and immunotherapy for the duration of the project. Both Dr Ewan and Dr Clark have undertaken multiple funded research projects for the UK Government (FSA-Food Standards Agency) continuously since 1999, with an excellent record for delivering milestones and objectives on time, resulting in publications and other outputs that have informed Agency policy. Dr Ewan and Dr Clark both serve as professional experts in allergy and contribute to National and European level Committees, UK Government Agencies and cross party reviews of allergy services and research and regularly review project funding proposals for the FSA. Dr Clark was recently expert advisor to the Committee of Toxicity to produce new advice for the Department of Health on the consumption of peanuts by high-risk mothers and infants in early life. Dr Sabita Islam is the laboratory research associate. She is a post-doctoral scientist who has worked in multiple projects examining the pathophysiology of food allergy and in particular the role of T lymphocytes in the resolution of food allergy. Dr Islam has great experience of the relevant cell isolation and culture techniques and flow cytometry. She has an excellent track record for directly supervising phD students. We are very fortunate to have Dr Islam on our team, there are very few research associates with such a detailed working knowledge of immunology with relevance to allergy. Dr Islam will perform the laboratory assays and will undertake training and supervision of Dr Anagnostou for the scientific aspects of her PhD. Dr Ewan, Dr Clark, Dr Islam and Sr King have worked as a well established team over recent years with great success.

Dr Katherine Anagnostou is a clinical fellow in paediatric allergy at Addenbrooke's who has committed to a career track whose eventual aim is an academic career in Paediatric Allergy. Dr Anagnostou will be undertaking the day to day challenges / immunotherapy as well as laboratory research within the project leading to a PhD in Allergy. She has obtained her basic Paediatric Specialist Registrar Training and has spent over six months (full time) performing challenges and oral immunotherapy in the pilot study under Dr Clark's supervision, which has given us a significant head start in preparation for this trial. Sr Yvonne King is the research allergy nurse. She has

invaluable experience in trial administration, performing food challenges and up-dosing in peanut allergic children, and has worked with Dr Andrew Clark on the same projects for the past six years. Sr King will be responsible for study administration and performing nursing supervision during interventions. Dr Chris Palmer if the Founding Director of the Centre for Applied Medical Statistics, Cambridge. Consulting and collaborating since 1996 has led to over 200 publications co-authored by a CAMS statistician, while his personal tally since 1991 exceeds 50. Dr Palmer has served on the panel of statistical reviewers for The Lancet for well over a decade. He has acted as statistician to several study Data Monitoring Committees and Trial Steering Committees, including multinational trials in disease areas in paediatrics, cardiology and oncology.
We are currently running a five year study funded by the Food Standards Agency to examine the T lymphocyte phenotype changes which occur with natural resolution of egg allergy. This study also uses oral challenges to define the allergic status of each child, although there is no active intervention. Our experience has been invaluable in setting up the OIT pilot study and the current proposal. We have also been able to develop the mechanistic work required for the current proposal from our earlier work with egg allergens. The studies will run side by side without competition,

16. Service Users:
Feedback from the responses received after publication of our news article confirm that the issues presented in this study are important to service users, who universally expressed relief that an active treatment for peanut allergy was being studied. A statement by the national patient support group (Anaphylaxis campaign) reinforces this view: 'The [pilot] study led by Dr Andy Clark at Addenbrooke's is extremely positive and will be of real interest to the many people currently living with peanut allergy. The Anaphylaxis Campaign is fully supportive of this type of reputable research and is delighted to see such interesting results….the Anaphylaxis Campaign believes this is the most exciting piece of research to emerge since the Campaign was founded ...' [http://www.anaphylaxis.org.uk/]. Ian Stewart MP, chair of the All Party Group on Allergy wrote to members to draw their attention to our study, saying 'such rare breakthroughs in research on allergy will bring hope to many patients suffering from … peanut allergy' [full text in appendix 2]. We also sought the opinions of families participating in the pilot study to identify any problems with the service and feedback is ongoing. The Anaphylaxis Campaign executive has reviewed the current proposal, on behalf of their members and considers that the outcome measures appropriately address their needs. Further, David Reading of the Anaphylaxis Campaign will join the Trial Steering Committee.

17. Justification of support required: This is a clinical trial with frequent high-intensity clinical interventions which requires close clinical supervision by a doctor and nurse at all times, for safe conduct. Dr Anagnostou (Clinical research associate; 1.0 WTE) will supervise peanut challenges and immunotherapy doses; she will work towards a PhD within this project, examining clinical and immunological features of oral immunotherapy. Sr King (Grade G nurse; 1.0 WTE) will prepare and perform oral challenges and immunotherapy with Dr Anagnostou. Dr Islam is a post doctoral scientist (research associate 7; 1.0 WTE) and will develop and perform the immunological assays with Dr Anagnostou, prepare updosing and double-check challenge preparation. Dr Islam is also responsible for training and supervising the PhD student in laboratory technique. Dr Ewan is group leader and will provide overall supervision. All will contribute to manuscript preparation. Dr Chris Palmer (University Lecturer) is trial statistician and will prepare statistical reports and perform statistical analysis.

18. References
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Table 1 NCI-CTCAE attribution of adverse events

Code	Descriptor	Definition
		Unrelated Category
1	Unrelated	The adverse event is clearly not related to study participation.
		Related Categories
2	Unlikely	The adverse event is doubtfully related to study participation.
3	Possible	The adverse event may be related to study participation.
4	Probable	The adverse event is likely related to study participation.
5	Definite	The adverse event is clearly related to study participation.

Table 2 Project milestone and completion dates

Date of completion	Milestones
01/09/09	Obtain ethics approval
31/12/09	Recruit 104 participants
31/12/09	Complete pre-intervention Qol questionnaires
31/03/10	Publish protocol
31/09/10	Complete initial peanut challenges
31/09/10	Complete pre-intervention blood sampling
31/09/10	Complete group allocation
31/09/10	Annual progress report
31/09/10	Submit abstract to EAACI/BSACI/AAAAI conferences
15/04/11	Complete initial immunotherapy in active group
31/03/11	Complete waiting list period for control group
15/07/11	Complete second challenges
31/03/11	Complete 14d blood sampling
31/09/11	Submit abstract to EAACI/BSACI/AAAAI conferences
31/09/11	Annual progress report
15/01/12	Complete immunotherapy in control group
15/04/12	Complete third challenges
15/04/12	Complete post-intervention Qol questionnaires
15/04/12	Complete 150d blood sampling
15/04/12	Complete analysis of IgG/IgE
15/04/12	Complete analysis of PBMC proliferation and cytokine profile
15/04/12	Complete analysis of basophil data
15/04/12	Complete analysis of HR-1/2 data
15/04/12	Submission of abstract to EAACI/BSACI/AAAAI conferences
15/04/12	Draft manuscript for publication
15/04/12	Draft final report
01/0612	Submission of manuscript
01/06/12	Final report submission

Figure 1 Study design