STUDY PROTOCOL

PROTOCOL TITLE:
Pancake Oral Immunotherapy For Egg Allergy In Inducing Tolerance (POET)

PROTOCOL NUMBER:

PROTOCOL VERSION: 5.0
PROTOCOL DATE: 15 Dec 2022

PRINCIPAL INVESTIGATOR:
Dr Chong Kok Wee, Consultant, Allergy Service, Paediatric Medicine, KKH

Table of Contents

Contents

PROTOCOL SIGNATURE PAGE

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Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described trial in compliance with all stipulations of the protocol, regulations and ICH E6 Guideline for Good Clinical Practice (GCP)

Principal Investigator Name: __Chong Kok Wee_____

Principal Investigator Signature: _____

Date: _____

1 BACKGROUND AND RATIONALE

1.1 General Introduction

Food allergy is increasingly recognized as a growing public health burden, with prevalence of up to 10% of infants in some countries (1). Egg and cow's milk allergies are the two commonest food allergies affecting young children worldwide. In Singapore, egg allergy is the most common food allergy seen in children younger than 2 years old, with a prevalence of 1.8% at 12 months old (2). While most egg allergic patients naturally acquire tolerance to eggs by early to late childhood (about 50-73% by 6 years old) (3),(4), some continue to have persistent egg allergy as adolescents and adults.

Most children with egg allergy (~70%) tolerate eggs in the baked form (5). Heat processing reduces the allergenicity of food proteins, presumably by changing the conformation of heat labile proteins that results in loss of conformational epitopes. Allergen interaction with a food matrix, such as wheat, can also make epitopes less accessible and decreases allergenicity. There is evidence that baked egg tolerant patients appear to develop tolerance to all forms of egg earlier than those reactive to baked egg. There is also some suggestion that introducing baked egg into the diet of a baked egg tolerant patient may accelerate resolution of egg allergy.

Management of food allergy is primarily allergen avoidance; however, given the ubiquity of egg in food, strict avoidance is difficult and accidental reactions do occur. The psychosocial impact in the quality of life of food allergic patients, as a result of dietary restrictions, has been well-described. Oral immunotherapy (OIT) refers to feeding an allergic individual an increasing amount of an allergen in the attempt to induce desensitisation, sustained unresponsiveness (SU) or ideally oral tolerance. Desensitisation is defined as a temporary increase in the reaction threshold to an allergen while receiving active therapy while SU is the lack of clinical reaction to an allergen after active therapy has been discontinued for a period of time. Egg OIT using various forms of egg (pasteurized raw egg white, scrambled eggs, hard-boiled egg etc) has been successful in desensitizing egg-allergic children, with rates ranging from 55% to 100% (6). On the other hand, rates of SU following egg OIT have been much lower ranging from 28-50% although it has been postulated that longer durations of OIT may improve SU rates. Furthermore, the occurrence of frequent allergic reactions (including anaphylaxis) together with a treatment failure rate of around 20% has limited OIT largely to research settings.

The use of baked egg OIT in baked egg reactive children has been reported in very few studies (7),(8), with published rates of desensitisation to cooked/ lightly cooked egg (hard boiled/ scrambled) at ~50%. The safety profile of baked egg OIT in these studies were favourable, with all reactions recorded as mild and none requiring adrenaline. Using baked egg which is the least allergenic form of egg and presumably less immunogenic, as a form of OIT, can explain the lower rates of desensitisation as well as severe adverse events.

1.2 Rationale and Justification for the Study

The aim of this study is to assess the efficacy, safety and feasibility of using pancakes as a form of OIT in desensitising egg allergic children.

1.2.1 Rationale for the Study Purpose

OIT using raw/ cooked egg has good desensitisation outcomes but is associated with frequent and sometimes severe adverse events (anaphylaxis is not uncommon). OIT using baked egg is less effective at inducing desensitisation but has a better safety profile. The compliance to daily consumption of baked egg products (muffins/ biscuits) after a negative baked egg challenge in egg allergic patients has also been reported to be poor, secondary to taste fatigue in children and need for frequent baking. A study using baked egg OIT had 38% withdrawal due to difficulties in ingesting the baked egg product daily (8). Pancakes, traditionally described as a flat cake prepared from a starch-based batter containing egg and milk and cooked on a hot surface for 5-7 minutes, is likely to be less allergenic than cooked egg because of the wheat matrix but more allergenic than baked egg. To date, there are no published studies investigating the use of pancakes in egg OIT. We hypothesize that pancakes are more effective than baked eggs in inducing desensitisation and sustained unresponsiveness while reducing the risk of adverse events associated with egg OIT.

1.2.2 Rationale for Study Design

This is a randomised controlled study exploring the efficacy, safety and feasibility of a novel form of egg OIT using pancakes to induce desensitisation and sustained unresponsiveness in egg allergic patients. The control group receiving current standard care of egg avoidance, will be used to control for the incidence of natural resolution of egg allergy over the study period. Safety of OIT and its impact on quality of life measures will also be compared against the risk of accidental reactions among controls who continue strict egg avoidance. Allocation ratio of 2:1 (active:control) allows for more patients to be given a chance to receive OIT.

This is an open label study as we wish to assess a pragmatic approach to OIT with home preparation of pancakes and cookies using store bought eggs, in which blinding will not be feasible nor practical.

Subjects randomised to the active arm will undergo an open baked egg food challenge (FC) to determine their baseline baked egg tolerance. Baked egg tolerant (BET) patients will directly undergo OIT using pancakes. Baked egg reactive (BER) patients will commence OIT with baked egg (cookies), to first achieve desensitisation to baked egg before transitioning to pancakes. Desensitisation will be assessed by an open cooked egg FC at the end of treatment while SU will be assessed by an open cooked egg FC after stopping OIT for approximately 6 weeks.

Updosings for cookies will be done at home under the guidance of the study team based on the safety profile of other home-based baked egg OIT protocols (7),(8). Updosings for pancakes will be performed in the hospital as this has not been evaluated previously.

Subjects randomised to the control arm will continue strict egg avoidance and undergo an open cooked egg FC between 12 and 18 months.

1.2.3 Rationale for Study Population

Egg allergy is one of the commonest food allergies affecting children. Most published egg OIT trials include patients above 4-5 years of age as these patients are believed to have a lower chance of acquiring natural tolerance without intervention. Given that OIT may potentially accelerate tolerance acquisition in younger children, we have chosen to include subjects between 2-15 years old in this study. Furthermore, recruiting children of a wider age range will allow us to better assess the safety and feasibility of using pancakes for OIT in children of various ages, particularly the acceptability of pancake in the younger cohort.

1.2.4 Rationale for Doses Selected

The BET group will undergo a 4-dose initial escalation with pancakes in hospital (37.5mg, 75mg, 150mg, 300mg egg protein) to determine the home starting dose of pancake. Those reacting at 37.5mg or 75mg will not begin OIT with pancakes but instead begin with cookies, as the amount of pancake required to begin OIT in these patients will be too small for practical home measurement. The home starting dose will be 1 step below the dose tolerated during initial escalation to minimise risk of allergic reactions at home, particularly as the presence of co-factors may decrease reaction threshold. Dose increments during the pancake build-up phase will occur every 4 weeks until a target maintenance dose of 2500mg egg protein is achieved. This maintenance dose was selected for reasons of safety and practicality as this translates to 1 pancake which is easier to consume on a daily basis.

The BER group who tolerate at least 3mg of muffin in the baseline baked egg FC will undergo initial escalation with cookies in hospital. The first dose to be administered during the initial escalation will be individualised based on the outcomes of the baseline baked egg FC while the home starting dose will be the last dose tolerated during initial escalation. Subsequent dose increments will occur every 2 weeks at home until a target of 2500mg egg protein (the amount tolerated in a baked egg FC) is tolerated before transitioning to pancake OIT.

2 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

Oral immunotherapy (OIT) using pancake is safe and effective in desensitising egg-allergic children to cooked egg.

2.2 Primary Objectives

To evaluate the efficacy of pancake OIT in inducing desensitisation in egg-allergic children.

2.3 Secondary Objectives

1. To evaluate the efficacy of pancake OIT in inducing sustained unresponsiveness in egg-allergic children.
2. To evaluate the safety and feasibility of using pancake as OIT for egg-allergic children.
3. To compare immunological outcomes (skin prick test to egg white, serum IgE to egg white, ovomucoid, ovalbumin) at baseline and end of OIT program.
4. To assess the impact of OIT on health-related quality of life.
5. To compare the immune cell profiles at baseline and end of OIT program.
6. To identify the mechanistic basis of resistance, desensitisation and SU to OIT.

2.4 Potential Risks and Benefits:

2.4.1 Potential Risks

Allergic reactions are expected during baseline challenges to cooked egg and baked egg and they also frequently occur through the course of OIT. The risk of experiencing an allergic reaction is higher in those undergoing OIT than those who are avoiding the allergen (9). Most adverse events are expected to be mild and local reactions (itch in mouth, rashes, abdominal pain or nausea). However, systemic reactions including anaphylaxis can occur, either in the hospital during FC/ updosings or with home doses.

It has been reported that small number of patients (~2%) undergoing OIT can develop eosinophilic oesophagitis (EoE), which is almost always reversible by stopping OIT, without long-term sequelae.

Risks from venepuncture are low and mainly associated with transient discomfort which can be mitigated by the use of local anaesthetics.

2.4.2 Potential Benefits

OIT aims to induce desensitisation in food allergic patients, hence reducing the risk of an (unpredictable) allergic reaction to egg through accidental exposure. This is despite the increase in frequency of allergic reactions during OIT, which are often controlled and predictable. This can reduce parental anxiety when they are eating out or travelling, potentially improving their quality of life. Successful desensitisation to egg can also broaden their dietary variety by introducing egg-containing foods and also reduce social limitations such as exclusion from birthday parties in the younger age group.

Participating in the study will contribute to existing evidence of OIT as a potential disease-modifying intervention in food allergic patients.

3 STUDY POPULATION

3.1 List The Number and Nature of Subjects to be Enrolled.

Children aged 2-15 years with current egg allergy will be identified from the KKH Paediatric Allergy Service. A total of 33 egg-allergic patients will be recruited and randomised 2:1 to active (n=22) or control (n=11) groups.

3.2 Criteria for Recruitment and Recruitment Process

Potential participants in outpatient clinics will be approached by the study team and existing patients may be contacted by their attending allergists (part of the study team) to be invited to participate in this study. Study Participant Information Sheet and Consent Form will be provided to participants prior to screening visit. The consent form will describe the purpose of the study, the procedures to be followed and the risks and benefits of participation. Patients contacted through phone/email, who are keen to participate will have a separate screening visit. Informed consent will be obtained from parents/ legal guardian, and assent will be obtained from children 6 years old and above prior to gathering study specific personal information and performing study specific procedures (eg. skin prick tests, food challenge).

3.3 Inclusion Criteria

Subjects must meet all the inclusion criteria listed below to participate in this study.

a. Age 2-15 years at time of consent.

b. Allergic to 4.443g egg protein or less, at baseline egg open food challenge OR

   Convincing clinical reaction to egg within past 6 months (or failed a clinical egg food challenge in last 6 months) AND evidence of current sensitization (positive SPT or egg-specific IgE performed within the last 3 months)

c. Written, informed consent of parent/legal guardian and patient assent (for those 6 years old and above).

3.4 Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from participation.

- Required previous admission to an intensive care unit for management of an allergic reaction.
- Children with a past history of egg allergy currently consuming egg-containing products other than extensively-heated egg in baked foods (e.g. biscuits, cakes).
- Developed severe anaphylaxis to egg or egg-containing products requiring more than 2 adrenaline auto-injectors or intravenous adrenaline infusion.

- Poorly controlled asthma within the previous 3 months (as defined by clinician judgement with reference to the ICON guidelines).
- Moderate-severe eczema despite appropriate use of emollients (eczema is not otherwise an exclusion criteria).
- Clinically significant chronic illness (other than asthma, rhinitis or eczema).
- History of symptoms of eosinophilic oesophagitis, irrespective of cause.
- Undergoing specific immunotherapy to another allergen and within the first year of treatment.
- Receiving anti-IgE therapy, oral immunosuppressants, beta-blocker or ACE inhibitor.
- Pregnancy.
- Unwilling or unable to fulfil study requirements.

3.5 Subject Replacement

Subjects who drop out will not be replaced.

4 STUDY DESIGN

This is a single site, open label interventional, randomised controlled trial evaluating the efficacy, safety and feasibility of pancake OIT, in inducing desensitisation and sustained unresponsiveness in egg allergic children between 2-15 years old. Participants will be recruited from the Allergy Department at KK Women's and Children's Hospital and assessed for eligibility based on the inclusion/ exclusion criteria listed. Those fulfilling criteria will be invited for an open FC to cooked egg (hard boiled), to confirm the diagnosis of an egg allergy (TS). *Children with a history of convincing clinical reaction to egg or failed a clinical egg food challenge within the last 6 months AND evidence of egg sensitisation on either skin prick test (SPT) or egg-specific IgE within the last 3 months will be exempted from this baseline FC. Those with a confirmed diagnosis of egg allergy will then be randomised 2:1 to active OIT or control.

Subjects randomised to the active arm will undergo a baked egg FC on a separate day in the unit (T0). This will be in the form of muffin baked by parents using standardised recipes (2 eggs in 6 muffins). *Children who have failed a baked egg FC within the last 6 months can be exempted from this baked egg FC. Depending on the outcome of the baked egg FC, they will be allocated to baked egg tolerant (BET) group (tolerating 2.5g baked egg) or baked egg reactive (BER) group (failing baked egg FC at any stage).

The BET group will undergo a 4-dose initial escalation with pancakes in hospital (37.5mg, 75mg, 150mg, 300mg egg protein) to determine the home starting dose of pancake (T1P). Those reacting at 37.5mg or 75mg will not begin OIT with pancakes but instead begin with cookies (Table 6 Step 11). The starting pancake dose will be administered at home daily for 4 weeks (minimum 3 weeks) before the next updosing to be performed in hospital. When

participants have successfully reached the target maintenance dose (Table 5 Step 7), they will remain on this maintenance dose for a minimum of 16 weeks (or total OIT duration of 18 months, whichever earlier).

The BER group who tolerate at least 3mg of muffin in the baseline baked egg FC will undergo initial escalation with cookies in hospital (T1C). The first dose to be administered during the initial escalation will be individualised based on the outcomes of the baseline baked egg FC while the home starting dose will be the last dose tolerated during initial escalation. The starting cookie dose will be administered daily at home for a minimum of 2 weeks. Subsequent updosings will be done at home on a 2-weekly basis. When participants have successfully reached the target dose (Table 6 Step 11), they will remain on this dose for a minimum of 2 weeks before undergoing pancake initial escalation to begin pancake OIT (T2P). Those reacting at 37.5mg or 75mg will not begin OIT with pancakes but instead resume cookie OIT Step 11 for another minimum 4 weeks before re-attempting pancake initial escalation.

All participants who have completed at least 16 weeks of maintenance pancake OIT will undergo an open cooked egg FC at the end of treatment to assess for desensitisation (T3). Participants who tolerate 4443mg of cooked egg and deemed successfully desensitised will then stop OIT for approximately 6-8 weeks prior to returning for a repeat cooked egg FC to assess for sustained unresponsiveness (T4).

Participants who tolerate 4443mg of cooked egg at SU FC will be allowed ad libitum cooked egg consumption post study. Participants who develop allergic reactions at SU FC will resume previous maintenance dose.

Control subjects will receive the current best standard care, i.e. strict egg avoidance with provision of rescue medication and training. They will then undergo an open cooked egg FC between 12 and 18 months to assess for cooked egg tolerance.

Approximate time to complete study recruitment is 12 months. Total OIT duration from initial escalation to desensitisation FC is a minimum of 12 months and maximum of 18 months.

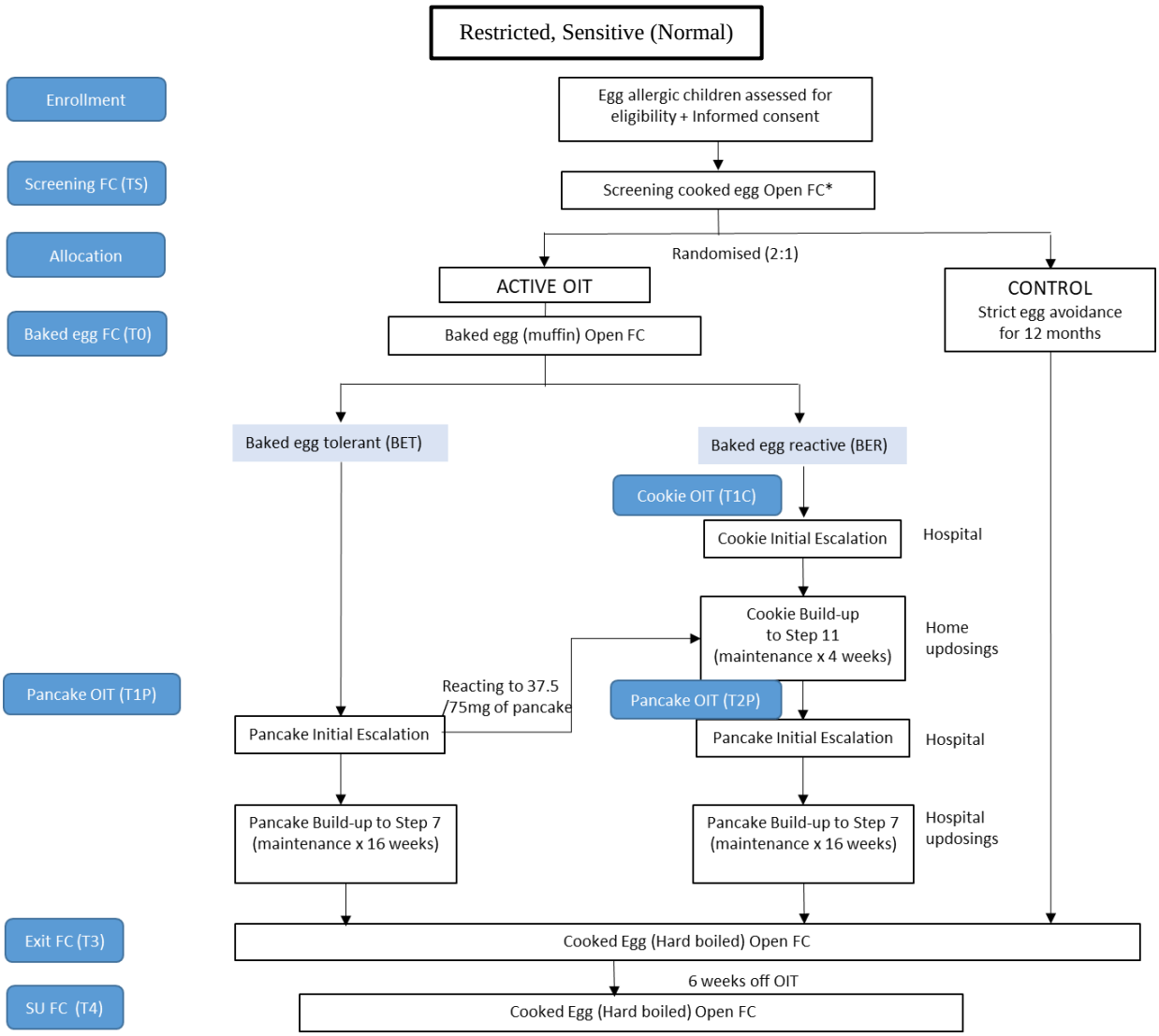

Figure 1: POET Study Design

4.1 Randomisation and Blinding

OIT outcomes have been reported to be affected by a range of variables, including age, baseline egg-specific IgE, baseline reaction threshold and the presence of asthma (10). It is therefore essential to maintain balance between treatment groups with respect to these predictors. However, stratified randomisation using several variables is not effective in small trials. Minimisation is a valid alternative to ordinary randomisation, and has the advantage that there will be only minor differences between groups in baseline variables which are used in the allocation process (11). With minimisation, the treatment allocated to the next participant enrolled in the trial depends on the characteristics of those participants already enrolled, with an element of weighted randomisation – typically 80% chance – of each participant getting the allocation that minimises any imbalance. Randomisation with 2:1 allocation ratio will be performed using an online free, open-source, desktop minimization program with a random element using a weighting probability of 0.8 (MinimPy Program 0.3).

Baseline variables included in the minimisation will be:

- Age (<5, ≥5 years)
- Egg-specific serum IgE (<10 kU/L, ≥10 kU/L)
- Asthma on inhaled corticosteroids (No, Yes)

This is an open label study and blinding will not be performed.

4.2 Contraception and Pregnancy Testing

Females of childbearing age will be tested for pregnancy using serum HCG if they are reported to be sexually active.

4.3 Study Visits and Procedures Overview

There will be 9-10 hospital visits for the BET group, 10-11 hospital visits for the BER group over the entire study duration. There will be 2 hospital visits for the control group.

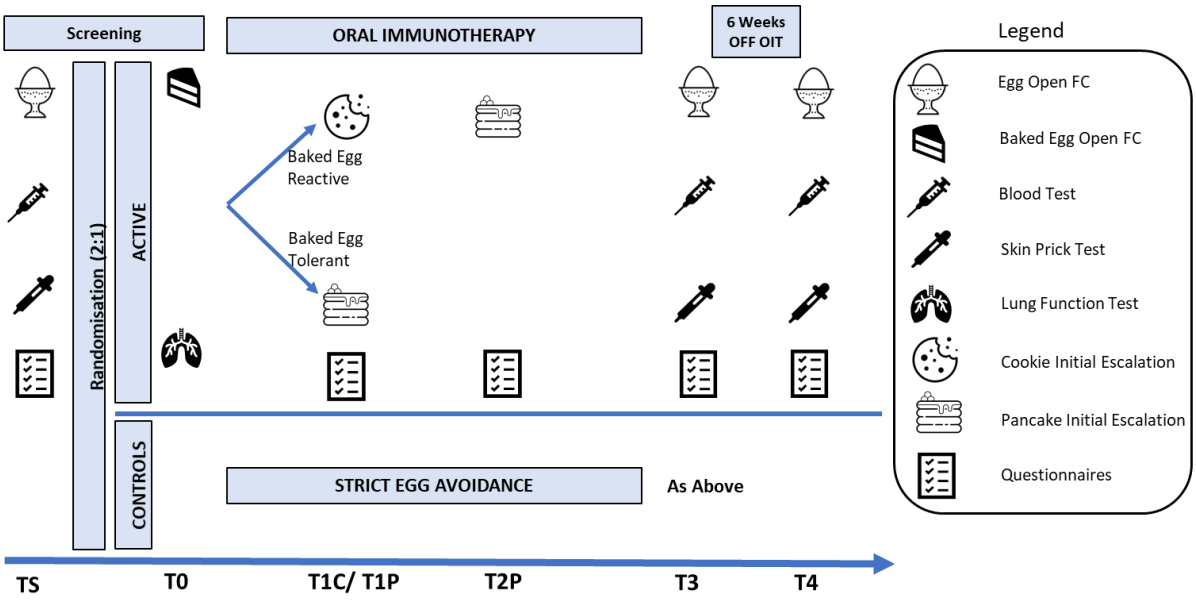

Figure 2: POET Study Timeline

Study Timepoints	TS	T0	T1C		T1P/ T2P		T3	T4
	Screening & Cooked Egg FC	Baked Egg FC	Cookie Initial Escalation	Home Cookie Build-up	Pancake Initial Escalation	Hospital Pancake Build-up	Exit Cooked Egg FC	SU FC
Informed consent	X							
Medical history	X							
Concomitant medications	X	X	X		X	X	X	X
Adverse events		X	X	X	X	X	X	X
Physical exam	X	X	X		X	X	X	X
Spirometry		(X)					(X)	
Asthma control test	X	(X)					(X)	
SCORAD	X							
POEM	X							
Skin Prick Test	X*						X	X
Blood test	X*						X	X
HRQL Assessments	X		X		X		X	X

Table 1: Study Procedures

(X) may be performed, depending on clinical need; X* - Results from SPT/ IgE performed within 3 months prior will be used and need not be repeated

X are procedures applicable to control subjects

FC – Food Challenge; SU – Sustained Unresponsiveness

4.3.1 Screening Visits and Procedures

Informed consent and screening procedures listed below can be performed on the day of patient's scheduled allergy outpatient visit or on the day of screening FC, or at a separate clinic visit. Once the subject fulfils all eligibility criteria, he/she will be randomised 2:1 to active OIT or control arms.

The following will take place at the first screening visit (TS):

- Written informed consent and participant assent.
- Clinical history and directed physical examination.
- For children with asthma, current asthma status will be assessed by the clinician and completion of a validated asthma control test.
- For children with eczema, eczema will be assessed using the SCORAD system (which provides an objective measure of eczema severity) as well as the POEM questionnaire, a validated patient-based symptom assessment.

- Skin prick test (SPT) to commercial extracts of egg (accepted if it was performed within 3 months prior to screening visit).
- Blood test - Serum IgE to egg white, ovomucoid and ovalbumin (accepted if it was performed within 3 months prior to screening visit) and immunoprofiling tests.
- Health-related quality of life (HRQL) assessment.
- Females of childbearing age will be tested for pregnancy using serum HCG if they are reported to be sexually active.
- Screening open FC to cooked egg. (*Except those with convincing clinical reaction to egg or failed a clinical egg food challenge in last 6 months) AND evidence of current sensitization - positive SPT or egg-specific IgE performed within the last 3 months)

Subjects assigned to the active arm will undergo an open baked egg FC in KKH outpatient Allergy unit. OIT should commence (initial escalation) at soonest possible date after baked egg FC.

Subjects assigned to the control arm will have baseline SPT and blood test (serum IgE to egg white, ovomucoid, ovalbumin) performed during screening visit.

Screening Food Challenge to Cooked Egg		
Dose	Egg Protein (mg)	Equivalent serving of cooked egg (g)
1	3	0.02
2	10	0.08
3	30	0.24
4	100	0.8
5	300	2.4
6	1000	8
7	3000	24
Cumulative dose	4443	35.54

Table 2: Dosing protocol for Screening Open FC to cooked egg

For subjects who pass the screening FC (ie. tolerate up to dose 7), the investigator may elect to give participant an 8th dose - 3000g egg protein, to confirm tolerance. Subjects who tolerate up to dose 7 would have failed screening and inclusion criteria.

4.3.2 Study Visits and Procedures

FC visits, initial escalation visits and pancake updosing visits will be conducted in the Allergy Unit, with staff fully trained in the management of allergic reactions including anaphylaxis and with emergency equipment and medication available.

4.3.2.1 Baked Egg Open Food Challenge (T0)

All eligible participants assigned to the active arm will undergo a separate day visit for baked egg FC (T0). This should be performed at soonest possible date after consent and screening FC. The baked egg FC will be in the form of muffin baked by parents using standardised recipes (2 eggs in 6 muffins).

The following will take place at the T0 visit (for active arm):

- Spirometry for subjects 8 years old and older, with a diagnosis of asthma/ suspected asthma. A recent spirometry (performed within the past 6 months) is acceptable if the control has been stable and there is no clinical concerns.
- Baked Egg Open Food Challenge (*Children who have failed a baked egg FC within the last 6 months can be exempted from this baked egg FC.)

Baked Egg (2 eggs in 6 muffins) Food Challenge		
Dose	Egg Protein (mg)	Equivalent serving of muffin (g)
1	3	0.07
2	10	0.24
3	30	0.72
4	100	2.4
5	300	7.2
6	1000	24
7	1057	25.4
Cumulative dose	2500	1 muffin

Table 3: Dosing protocol for Baked Egg Open FC

4.3.2.2 Pancake Initial Escalation Phase (T1P)

The baked egg tolerant (BET) group will undergo a 4-dose initial escalation with pancakes in hospital on a separate day (T1P) to determine the home starting dose of pancake. This should be performed at soonest possible date after baked egg FC.

Pancake Initial Escalation	Pancake Recipe A – 1 egg in 6 pancakes		If dose tolerated, commence build-up at:
Time Point (min)	Pancake amount	Egg Protein (mg)	
0	~1/32	37.5	NA*

20	1/16	75	Step 1: 37.5mg
40	1/8	150	Step 2: 75mg
60	1/4	300	Step 3: 150mg

Table 4: Dosing protocol for Pancake Initial Escalation

*Those reacting at 37.5mg or 75mg will not begin OIT with pancakes but instead begin with cookies (Table 6 Step 11).

BET subjects will commence pancake initial escalation at 37.5mg with increments every 20-30 minutes to a maximum of 300mg unless symptoms develop. The dose tolerated is one which results in no more than mild transient (not lasting more than 20 minutes) symptoms (e.g. itchy mouth) - transient Grade 1 symptoms based on the CoFAR specific grading symptoms for allergic reactions (Table 8). The home starting dose will be one step below the tolerated dose during initial escalation to minimise risk of allergic reactions at home, particularly as the presence of co-factors may decrease reaction threshold.

4.3.2.3 Pancake Build-Up Phase

Each dose increment for pancake will be performed in the Allergy Unit under medical supervision, to confirm tolerance before the new dose is given at home daily for 4 weeks (minimum 3 weeks). Mild transient symptoms (e.g. itchy mouth) to the dose - transient (not lasting more than 20 minutes) Grade 1 symptoms (Table 8) - might be acceptable. If the updosing is not tolerated (i.e. more than mild transient symptoms), the participant will stay on the previously tolerated dose for another 4 weeks (minimum 3 weeks) before returning for repeat updosing.

Pancake Build-up Phase Protocol	Pancake Recipe A – 1 egg in 6 Pancake Recipe B – 2 eggs in 6			Min. no. of months on this step	Cumulative no. of months (minimum)
STEP	Pancake Recipe	Pancake amount	Egg Protein (mg)		
1	A	1/32	37.5	1	1
2	A	1/16	75	1	2
3	A	1/8	150	1	3
4	A	1/4	300	1	4
5	A	1/2	625	1	5
6	A	1	1250	1	6
7	B	1	2500	4	10

Table 5: Pancake Build-up Phase Protocol

When participants have successfully reached the target maintenance dose (Step 7), they will remain on this maintenance dose for a minimum of 16 weeks (or total OIT duration of 18 months, whichever earlier).

4.3.2.4 Cookie Initial Escalation (T1C) and Build-up Phase

The baked egg reactive (BER) group who tolerate at least 3mg of muffin will undergo initial escalation with cookies under medical supervision in the hospital on a separate day (T1C) to determine the home starting dose of cookie. This should be performed at soonest possible date after baked egg FC. The first dose to be administered during initial escalation will be individualised based on the outcome of the baseline baked egg FC. The NOAEL (No Observed Adverse Event Level) – defined as highest dose triggering none or transient Grade 1 symptoms) will be used for this purpose. Subsequent increments will be administered every 20-30 minutes and the maximum number of doses to be administered during the initial escalation is stipulated in Table 6 below. If a given dose triggers symptoms that causes discomfort to the participant or is deemed significant by the research team, no further doses will be administered on that day. Participants will be observed for at least one hour after dosing (2 hours if participant has previously experienced a delayed reaction). Participants will be instructed to have the last well-tolerated dose at home daily for a minimum of 2 weeks, until the next updosing is performed.

Subsequent updosings will be performed at home by the parents once every 2 weeks. The study team will conduct a phone call 2 weeks after the initial escalation and then monthly phone calls, to check on the progress of home updosing, ensure compliance and review any adverse events. When participants successfully reach the target dose (Step 11), they will remain on this dose for a minimum of 2 weeks before undergoing initial escalation with pancake (T2P). Subjects who react at 37.5mg or 75mg during pancake initial escalation will not continue with pancake OIT but instead resume cookie OIT Step 11 for a minimum of another 4 weeks before re-attempting pancake initial escalation.

Cookie Initial Escalation (IE) and Build-up Phase Protocol						
NOAEL at Baseline BE FC (mg)	STEP	Cookie Recipe	Cookie Amount	Egg Protein (mg)	Max Egg Protein at IE (mg)	Max no. of doses at IE
3	1	C	1	1.25	5	3
-	2	C	2	2.5	-	-
-	3	C	4	5	-	-
10	*3.5	D	0.5	6.25	37.5	3
30	4	D	1	12.5	75	3
-	5	D	3	37.5	-	-
100	6	E	1	75	150	2
300	7	E	2	150	300	2
-	8	E	4	300	-	-
1000	9	F	1	625	1250	2
-	10	F	2	1250	-	-
-	11	F	4	2500	-	-

Table 6: Cookie Initial Escalation and Build-up Phase Protocol

* Step 3.5 is only for initial escalation for subjects with NOAEL of 10mg

Egg Content in Cookie Recipes:
C: 1ml egg in 100 cookies (1.25mg egg protein per cookie)
D: 10ml egg in 100 cookies (12.5mg egg protein per cookie)
E: 1 egg in 100 cookies (75mg egg protein per cookie)
F: 1 egg in 12 cookies (625mg egg protein per cookie)

4.3.2.5 Home Instructions & Checklist

Prior to discharge post initial escalation (pancake or cookie), each family will be provided with:

- A symptom advice sheet including instructions in the prompt recognition of allergic reactions.
- A food allergy action plan. Subjects and their families will be instructed in the use of adrenaline auto-injectors, antihistamines and salbutamol (if known to be asthmatic). The expiry date of medications will be checked, and a prescription for replacements provided if needed.
- A daily dosing diary and instruction sheet which will include the following:
    - Contact information for the team, which will include a dedicated mobile phone number with 24/7 access to a member of the medical team for urgent queries, and an email address which will be checked daily by the research team from Monday to Friday for non-urgent queries.
    - Information to GP / Children's Emergency in writing that the patient is undergoing OIT and that allergic reactions may occur during administration.
    - Instructions on study dose administration including the dose to give their child and advice to avoid intense physical exercise up to 2 hours before and after the dose intake. Subjects will be asked to log all doses taken at home and any resulting adverse events. In the event of any symptoms, the family needs to contact the study team for advice and record all symptoms, duration, timing in relation to immunotherapy dose and any exacerbating factors (e.g. exercise, excessive tiredness, viral illness
- Dietary advice on egg avoidance (See Section 6.4).

All control subjects will receive a food allergy action plan, instructions on use of adrenaline auto-injectors, antihistamines and salbutamol if needed, as well as dietary advice on strict egg avoidance.

4.3.2.6 Desensitisation (DS) Open Food Challenge (T3)

All participants who have completed at least 16 weeks of maintenance pancake OIT (Table 5 Step 7) will undergo an open cooked egg challenge at the end of treatment (T3) to assess for desensitisation. This is subjected to a total OIT duration (from initial escalation to exit challenge) of a minimum 12 months and maximum 18 months. All control subjects will undergo an open cooked egg challenge between 12 and 18 months (T3).

Desensitisation Open Food Challenge to Cooked Egg		
Dose	Egg Protein (mg)	Equivalent serving of cooked egg (g)
1	3	0.02
2	10	0.08
3	30	0.24
4	100	0.8
5	300	2.4
6	1000	8
7	3000	24
Cumulative dose	4443	35.54

Table 7: Dosing protocol for DS Open FC to cooked egg

Successful desensitisation is defined as passing the FC to cooked egg, tolerating 4443mg of egg protein.

4.3.2.7 Sustained Unresponsiveness (SU) Food Challenge (T4)

Desensitised subjects in the active arm (tolerating 4443mg of cooked egg at T3) will be asked to stop OIT and all baked egg products (if they had been taking them) for approximately 6-8 weeks prior to returning for a repeat FC to assess for sustained unresponsiveness. The dosing protocol for SU FC will be the same as that of DS FC (Table 7).

Participants who tolerate 4443mg of cooked egg at SU FC will be allowed ad libitum cooked egg consumption post study. Participants who develop allergic reactions at SU FC will resume previous maintenance dose.

4.3.2.8 Clinical assessment and procedures during open FC (screening FC, baked egg FC, DS FC, SU FC)

All challenges will be conducted in the Allergy outpatient unit, with staff fully trained in the management of allergic reactions including anaphylaxis. Emergency equipment (including oxygen and suction) and medication will be checked beforehand, as per local protocol and international criteria for performance of FC.

All subjects will be assessed prior to FC to determine suitability for a challenge, as follows:

- No intercurrent illness
- No exacerbation in allergic symptoms (eczema, asthma, food allergy) in the preceding week.
- No short-acting β2 agonists used in the past 12 hours
- No recent antihistamine exposure (e.g. cetirizine, loratadine) in the past 48 hours (72 hours for fexofenadine, 5 days for long-acting antihistamine (e.g. chlorphenamine, desloratadine).

• No oral steroids have been taken in the past 2 weeks

• Baseline observations (temperature, blood pressure, heart and respiratory rate, oxygen saturations,) within the normal range.

• Baseline physical examination must not reveal any significant acute findings.

The clinical team may elect to secure intravenous access by cannulation as an additional safety precaution at challenge. This will allow treatment to be easily and rapidly administered in the event of a significant allergic reaction, and also allows blood to be collected without further venepuncture.
Participants will be monitored including heart rate, blood pressure and oxygen saturations. Clinical manifestations will be assessed throughout the challenge.

Dose intervals should be between 20-30min but may be extended up to one hour at the investigator's discretion, where the participant may be experiencing an evolving reaction (as a safety measure, to limit the dose which might in turn moderate the symptoms experienced at challenge). A 2-hour observation period will follow the last dose to monitor for signs of reaction. For safety reasons, families will be asked to provide their child with a snack/ lunch to eat one hour after the last dose has been given or the challenge has been stopped due to reaction. Participants will then be observed for a second hour prior to leaving the unit.

Symptoms will be monitored during the challenge, with detailed assessment prior to each challenge dose, according to Figure 3.

• Green symptoms: Not generally sufficient to consider a challenge positive.

• Orange symptoms: Symptoms that recur on 3 doses or persist (e.g. 40 minutes) are more likely indicative of a reaction than when such symptoms are transient and not reproducible. 3 or more scoring areas in orange, more likely represent a true response.

• Red symptoms: A single red symptom is likely to indicate a true response, and if present the challenge should be halted.

The outcome of the challenge (positive or negative) and the apparent eliciting threshold dose will be recorded in the participant record. Challenge outcome will be determined as per the PRACTALL Consensus criteria, as shown in Figure 3; where possible, challenges should be stopped on the basis of at least one objective symptom.

Any allergic reaction occurring during in-hospital food challenge will be managed according to our unit's established protocol.

I. SKIN

A. Pruritus

0 = Absent

1 = Occasional scratching

2 = Scratching continuously for >2 minutes at a time

3 = Hard continuous scratching causing excoriations

B. Urticaria/Angioedema

0 = Absent

1 = < 3 hives, or mild lip oedema

2 = < 10 hives but >3, or significant lip, tongue or facial oedema

3 = Generalized involvement

C. Rash

0 = Absent

1 = Few areas of faint erythema

2 = Areas of erythema

3 = Generalized marked erythema (>50%)

II. UPPER RESPIRATORY

A. Sneezing/Itching

0 = Absent

1 = Rare bursts, occasional sniffing

2 = Bursts <10, intermittent rubbing of nose/eyes or frequent sniffing

3 = Continuous nasal/eye itch, periocular swelling and/or long bursts of sneezing, persistent rhinorrhoea

III. LOWER RESPIRATORY

A. Wheezing

0= Absent

1 = Expiratory wheeze on auscultation

2 = Biphasic wheeze

3 = Use of accessory muscles, audible wheezing

B. Laryngeal

0= Absent

1 = >3 discrete episodes of throat clearing/cough, or persistent throat tightness/pain

2 = Vocal hoarseness, frequent cough

3 = Stridor

IV. GASTROINTESTINAL

A. Subjective Complaints

0 = Absent

1 = Complaints of nausea or abdominal pain, itchy mouth/throat

2 = Frequent c/o nausea or pain with normal activity

3 = Notably distressed due to GI symptoms with decreased activity

B. Objective Complaints

0 = Absent

1 = 1 episode of emesis

2 = 2-3 episodes of emesis or diarrhoea or 1 of each

3 = >3 episodes of emesis or diarrhoea or 2 of each

V. CARDIOVASCULAR/NEUROLOGIC

0 = normal heart rate or BP for age/baseline

1 = Subjective symptoms (weak, dizzy), or tachycardia

2 = Drop in mean BP of >20% from baseline, or significant change in mental status.

3 = Cardiovascular collapse, signs of impaired circulation (unconscious)

Figure 3: PRACTALL consensus stopping criteria for food challenges

4.3.2.9 Quality of Life Assessments

The impact of OIT on participants' health-related quality of life (HRQL) will be assessed both from parents' and participants' perspective using disease-specific validated "food-allergy quality of life questionnaire" (FAQL-Q) in its parental, child and teenager forms. The FAQLQ-PF (parent form) will be administered to all parents; FAQLQ-CF (child form) administered to patients age 8-12 years at commencement of study; FAQLQ-TF (teenager form) administered to patients age 13-18 years at commencement of study. To ensure the ability to compare responses consistently during the 12 months of OIT, children who are age 12 at study commencement will be asked to complete the Teenage version unless there are difficulties with comprehension which preclude this.

These will be performed for subjects in the active arm at the following points during the study:

i. baseline, at screening (TS).
ii. prior to cookie initial escalation (T1C).
iii. prior to pancake initial escalation (T1P/ T2P)
iv. prior to the DS FC (T3).
v. prior to SU FC (T4).

Subjects in the control arm will be assessed at baseline (TS) and prior to DS FC (T3).

4.3.2.10 Laboratory Assessments

The development of tolerance in immunotherapy is associated with several immunological changes, including decreases in egg-specific IgE levels with concomitant increases in IgG4 levels. Serum IgE to egg white, ovomucoid and ovalbumin will be performed at baseline screening (TS) and at the end of study (T3) for all subjects and additionally for active subjects who have a visit at T4.

Other than serum immunoglobins assay, 3 mL of Ethylenediaminetetraacetic acid anti-coagulated blood will be concurrently collected (TS, T3 and if applicable, T4) and sent to a core laboratory for peripheral blood mononuclear (PBMC) preparation. We expect 3 mL of blood to yield approximately 6 million PBMCs. These following experiments will be performed:

i. DNA-human leukocyte antigen (HLA) genotypes: DNA will be isolated from 0.2 mL of blood to perform HLA typing to determine the appropriate candidates for major histocompatibility complex (MHC) tetramers analysis and genome sequencing if new data arises that indicates the importance of other non-HLA genetic variants for allergy manifestation.
ii. Multi-parametric immune cell profiling: We will utilise multi-parametric cytometry to holistically characterise the immunome and determine the differences between non-responders and those with SU to identify immune signatures that confer resistance to OIT. Absolute counts of B, T, natural killer and dendritic cell subsets will be characterised.

iii. Transcriptomic analysis: This will be done either at the immune cell subset or single cell level depending on the immune cell profiling data to identify the pathways responsible for the differences in response to OIT.

iv. Multi-parametric cytokines/chemokines profiling: This will be done on the plasma stored after PBMC preparation if the cytometry data indicates the involvement of specific cytokines/chemokines in allergy manifestation.

4.3.3 Final Study Visit:

The final study visit will be on the day of DS challenge (T3) for controls and for active subjects who did not achieve desensitisation. The final study visit will be on the day of SU challenge (T4) for those successfully desensitised at T3.

4.3.4 Post Study Follow up and Procedures

Subjects will be followed up by the Allergist in the clinical service and the monitoring and treatment of adverse events will be done during regular outpatient visits with the allergy team.

4.4 Discontinuation/Withdrawal

4.4.1 Discontinuation Criteria

Subjects will be free to withdraw from the study at any time without affecting their future medical care.

The safety of study participants is paramount: subjects who are assessed as having anaphylaxis to OIT dose at home but who have not administered IM adrenaline as per the management plan will have further updosing suspended until the family undergo retraining on the recognition and management of allergic symptoms.

A participant may be withdrawn from the study under the following circumstances:

• If, in the opinion of the study team, compliance with study procedures is suboptimal such that it compromises the patient's safety.

• If, in the opinion of the study team, further participation would adversely affect the participant's health.

• A child develops an exclusion criteria during the updosing phase of the study e.g. a new medication (which would normally be a contra-indication) is commenced.

• If consent is withdrawn or the subject fails to return for a study visit.

4.4.2 Discontinuation Visit and Procedures

All subjects withdrawn from study will be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the subject's condition becomes stable. Subjects withdrawn from study will be invited to a final discontinuation visit and if willing, the following will take place:

- Skin prick test (SPT) to commercial extracts of egg.
- Blood test - Serum IgE to egg white, ovomucoid and ovalbumin and immunoprofiling tests (4.3.2.10)
- Health-related quality of life (HRQL) assessment (4.3.2.9)

Withdrawn subjects will be offered continued follow up in the Allergy clinical service as per current clinical practice.

5.1 Trial Product (s)

5.1.1 Pancake & Cookie Recipes – A set of standardised baking tools including baking tray, measuring cup, measuring spoons, digital weighing scale, frying pan, syringes will be provided to subjects in the active arm, to ensure uniformity in the cookies and pancake.

Pancake Recipe

Recipe A		**Makes**	**Amount of pancake to be consumed**	**Egg protein (mg)**
Plain Flour	55g	6 pancakes (30ml or 1/8 cup per pancake)	1/32	37.5
Baking Powder	1 tsp		1/16	75
Baking Soda	1/8 tsp		1/8	150
Salt	1/8 tsp		¼	300
Sugar	15g		½	625
Butter	15g		1	1250
Milk	60ml			
Egg (65g)	1			
Vanilla Essence	½ tsp			
Recipe B		**Makes**	**Amount of pancake to be consumed**	**Egg protein (mg)**
Plain Flour	110g	6 pancakes (60ml or ¼ cup per pancake)	1	2500
Baking Powder	2 tsp			
Baking Soda	¼ tsp			
Salt	¼ tsp			
Sugar	30g			
Butter	30g			
Milk	120ml			
Egg (65g)	2			
Vanilla Essence	1 tsp			

Method

1. Microwave the butter for 20-30 seconds to melt it.
2. Combine the flour, baking powder, baking soda, salt and sugar in a large-sized bowl. Make a well in the centre and add the cooled melted butter, milk, egg(s) and vanilla essence.
3. Use a wire whisk and mix the wet and dry ingredients together until smooth. The batter should be thick and creamy in consistency.
4. Set the batter aside and allow to rest while heating up your pan.
5. Heat the non-stick pan over medium heat for 2 minutes then turn heat down to **low**.
6. Pour 30ml of Recipe A or 60ml of Recipe B onto the pan.
7. Cook for 3 minutes. Bubbles should appear on the surface and the edge of the pancake should peel off easily to reveal a golden underside.
8. Flip with a spatula and cook for another 3 minutes.
9. Repeat with remaining batter to make a total of 6 pancakes.

*tsp = teaspoon

Cookie Recipe

	Amount of egg used	**Makes**	**Amount of cookies to be consumed daily**	**Egg protein (mg)**
150g Plain Flour 100g Sugar 100g Butter/ Milk-free margarine ½ tsp Baking soda	Recipe C			
	1ml	100 cookies	1	1.25
			2	2.5
			4	5
	Recipe D			
	10ml	100 cookies	1	12.5
			3	37.5
	Recipe E			
	1 whole (65g)	100 cookies	1	75
			2	150
			4	300
	Amount of egg used	**Makes**	**Amount of cookies to be consumed daily**	**Egg protein (mg)**
100g Plain Flour 50g Sugar 50g Butter/ Milk-free margarine ¼ tsp Baking soda	Recipe F			
	2 whole (65g each)	24 cookies	1	625
			2	1250
			4	2500

Method

1. Preheat oven to **180°C.** Microwave the butter or milk-free margarine for 30-40 seconds to melt it.
2. Using a whisk, beat the melted butter with sugar until creamy. Add the egg and continue beating until just incorporated. Avoid beating the egg for too long as cookies will be stiff.
3. Add the flour and baking soda. Mix well together into a dough.
4. Line the square baking tray with baking paper. Roll the dough evenly onto the baking tray.
5. Bake in the oven for 15 minutes at **180°C.**
6. Once out of the oven, immediately cut into 10x10 squares (Recipe C-E) or 6x4 rectangles (Recipe F) before the cookies cool.

5.1.2 Food Safety

Raw Ingredients

- Eggs
    - *Salmonella* bacteria can be present on egg shells and the interior of normal-looking eggs.
    - Proper handling of eggs reduces the risk of *Salmonella* contamination. This includes storing eggs in the refrigerator until they are needed, and ensuring adequate personal hygiene and cleanliness of utensils during food preparation.

Cookies and Pancakes

- Baked and cooked products are subject to microbiological spoilage. This is influenced by factors such as storage temperature, relative humidity, initial contamination and moisture content of the product.
- Water activity, a_w, which is an indicator of moisture content, allows recognition of the spoilage and safety potential of baked goods. Cookies have a low moisture content (a_w <0.6) while pancakes have a high moisture content (a_w >0.85).
- Low moisture baked products such as cookies are less likely to be affected by microbiological spoilage. In high moisture cooked products like pancakes, almost all bacteria, yeasts and moulds are capable of growth.
- To control microbiological spoilage, the following strategies should be taken:
    - Cookies and pancakes should be cooled completely prior to storage.
    - Stored in airtight container.
    - Store at room temperature (if consumed within 2 weeks for cookies, and within 2 hours for pancakes) or in the freezer to prolong storage life.

5.2 Storage and Drug Accountability

Storage Requirements and Stability (Temperature, Duration)

Shelf Stable Ingredients

	Shelf (unopened)	**Shelf (opened)**
Plain flour	Use by date	6 months*
Sugar	Use by date	18 – 24 months
Oil	Use by date	3 – 5 months
Baking powder	Use by date	3 – 6 months

* Opened flour packets should be stored in air-tight containers.

Perishable ingredients

	Refrigerator
Eggs, in shell	3 – 5 weeks OR Use by date
Butter/ Milk-free margarine	1 – 2 months 6 months
Fresh cow's milk	Use by date (unopened) 3 days (opened)

Baked Products

	Room Temperature (~25°C)	Refrigerator (0 – 4°C)	Freezer
Cookies	2-3 weeks	2 months	8-12 months
Pancakes	2 hours	2 – 3 days	2 months

- Pancake batter must be cooked within 2 hours upon preparation.
- General safe food preparation and handling practices apply.

6 TREATMENT

6.1 Rationale for Selection of Dose

Current OIT regimens typically involve the daily consumption of an allergen, commencing at a very low dose followed by dose increments over several hours during initial escalation phase and periodically (usually every 2-4 weeks) during build-up phase until the target maintenance dose is achieved (12) This maintenance dose is then continued on a daily basis for months to years. There is considerable variation between studies regarding starting and target maintenance doses.

Egg OIT (using eggs in various forms such as lyophilized egg, dehydrated egg white, pasteurized raw egg white, lightly cooked eggs or hard-boiled eggs) has been reported with much heterogeneity in desensitisation rates ranging from 55% to 100%. Furthermore, the ability of current protocols to induce SU appear to be even more limited (~30%). It is possible that longer duration and higher doses of treatment resulting in higher cumulative dose of allergen may lead to higher rates of allergy remission but this would have to be balanced against the risk of allergic reactions. Safety of egg OIT, just like other OIT, is a concern with early discontinuation due to adverse events reported in 12.5%-36% of children (13).

Only 2 studies have evaluated using baked egg in OIT and both used different doses administered over different durations of time. In the study by Bravin et al, biscuits containing varying amounts of egg protein were administered to baked egg allergic subjects, starting at 125µg and increased daily over 60 days to a target maximum dose of 6.25g after which an open FC to 1 boiled egg was performed to assess for desensitisation. Of the 15 patients recruited, 12 (80%) completed the OIT program and all adverse reactions were mild. The second study by Bird et al. did not report the starting OIT dose but baked egg-allergic subjects underwent an initial escalation phase up to 125mg in hospital followed by home up-dosing to a maintenance dose of 3.8g which was continued for 1 year. This was followed by an open FC to 6g of lightly cooked egg to assess for desensitisation. Again, dosing was well tolerated with only 77 adverse reactions reported of 5605 total recorded doses (1.37% of total doses) and none requiring adrenaline.

Pancakes are expected to be less allergenic than cooked egg because of the wheat matrix but more allergenic than baked egg. In our study, all subjects assessed to be baked egg tolerant will have ingested and tolerated 2.5g of egg protein during the baseline baked egg open FC. BET subjects will hence commence pancake OIT at 37.5mg (1.5% of the 2.5g cumulative dose tolerated during the baked egg FC) with increments every 30minutes to a maximum of 300mg during the initial escalation phase (Table 4). The starting dose of the build-up phase will be 1 step below the dose tolerated during the initial escalation phase to minimise risk of allergic reactions at home, particularly as the presence of co-factors may decrease reaction threshold (see Section 6.4). Dose increments during the build-up phase will occur every 4 weeks (minimum 3 weeks) until a target maintenance dose of 2500mg is achieved (Table 5). Subjects will then remain on the daily maintenance dose for a minimum of 16 weeks or total OIT duration of 18 months, whichever is achieved earlier.

BER subjects who tolerate at least 3mg of muffin will commence OIT with cookies to first desensitise them to baked egg before transitioning to OIT with pancakes. The first dose to be administered during the initial escalation will be individualised based on the outcome of the baseline baked egg FC. Subsequent dose increments during the cookie build-up phase will occur every 2 weeks at home until the target of 2500mg egg protein (the amount tolerated in a baked egg FC) is tolerated. When participants successfully reach the target dose (Table 6 Step 11), they will remain on this dose for a minimum of 2 weeks before undergoing initial escalation with pancake.

All participants who have completed at least 16 weeks of maintenance pancake OIT (Table 5 Step 7) will undergo exit cooked egg FC to assess for desensitisation to cooked egg.

6.2 Study Drug Formulations

Standardised recipes for pancakes and cookies will be given to participants. Recipes are clearly labelled and clear updosing instructions (including change of recipe) are given to subjects at commencement of OIT, and with reminders by the study team at updosing intervals. All ingredients will be weighed where possible. Standardised frying pans and scoops will be used for pancakes. Doses of egg protein consumed daily will be determined by the quantity of pancake/cookie consumed following the dosing Tables 5 and 6.

6.3 Study Drug Administration

Initial escalation phase and all dose increments during the pancake build-up phase will be administered in the Allergy Unit under medical supervision. Dose increments during the cookie build-up phase will be performed at home as previous studies have reported very low risks of severe allergic reactions. This will reduce the number of hospital visits required. All doses (pancake and cookie) must be taken for a minimum of 1 week before the next updosing.

6.3.1 In the event of a dose-related allergic reaction

In the event of an allergic reaction to a dose of immunotherapy within 2 hours of a dose, either at updosing in hospital or after a dose given at home, families will be instructed to follow their Allergy Action Plan, and to contact the study team.

Anaphylaxis reactions at home

• Where a child is having objective symptoms consistent with possible anaphylaxis (e.g. wheeze, persistent cough, difficulty breathing, stridor or marked swallowing difficulties), families will be instructed to administer the adrenaline auto-injector pen, contact the study team on the Emergency number and call 995 for the child to be brought to KKH Children's Emergency for further evaluation and admission

Following resolution of symptoms, the previous tolerated dose will be given the next possible day, under medical observation.

Persistent/ bothersome symptoms at home

• For persistent symptoms that last ≥3days (within 2 hours of the immunotherapy dose) consisting of abdominal pain, rhinoconjunctivitis or skin symptoms (Table 8 Grade 1), the investigator should consider a dose reduction to the previous tolerated dose for at least 3 days or until symptoms have mostly resolved.

• In the event of other symptoms that prove bothersome to the child, the dose can be reduced at investigator's discretion.

• In cases of dose reduction due to persistent/ bothersome symptoms, the original dose should be resumed once symptoms are deemed to have mostly resolved. *Upon resumption of original dose, subjects should be on the dose for at least 1 week before the next updosing.*

• For recurrent symptoms where dose reduction has not on its own been effective, the investigator may elect to offer participants an oral second-generation antihistamine, prior to OIT dosing, to reduce allergic symptoms to immunotherapy doses. If started, the use of these medications should be minimized, and then discontinued at the earliest opportunity. Antihistamines should not be taken on the day of updosing.

Mild symptoms at home

• For mild skin symptoms, itchy mouth, rhinoconjunctivitis or mild abdominal symptoms (Table 8 Grade 1), reassurance to families will be provided and the current dose will be continued, with contact with the study team as needed.

6.3.2 In the event of omitted doses

• If 1-2 days of immunotherapy is missed, the subject can continue with the current dose at home with the next scheduled updose to proceed as planned

• If 3-7 days of immunotherapy are missed,

- for pancake build-up phase - the previous dose will be given until the subject can attend the Allergy unit for repeat updosing. *Upon resumption of original dose, subjects should be on the dose for at least 1 week before the next updosing.*
- for cookie build-up phase - the previous dose will be given for a week before repeat updosing at home. *Upon resumption of original dose, subjects should be on the dose for at least 1 week before the next updosing.*

• If more than 7 days of immunotherapy are missed, dosing should stop until the subject can attend the Allergy unit for repeat updosing of the previous dose.

6.3.3 In the event of sick days

Families will be requested to contact the study team in the event of an intercurrent illness, to discuss reducing the dose depending on symptoms and intensity.

In the event of subject having an intercurrent illness, the investigator may pre-emptively reduce the OIT to the previous dose for the duration of the illness. *Upon resumption of original dose, subjects should be on the dose for at least 1 week before the next updosing.*

6.3.4 In the event of dose reduction for other reasons

Subjects are expected to be compliant to doses and will be encouraged to adhere to the protocol. In the event the subject is unable to take the current dose for reasons other than allergic reactions and sick days (eg. refusal from taste fatigue), the previous dose will be given until the original dose is resumed. *Upon resumption of original dose, subjects should be on the dose for at least 1 week before the next updosing.* If the subject is taking less than the previous dose, subsequent dosing will be managed as per omitted doses (Section 6.3.2).

6.4 Specific Restrictions / Requirements

Subjects in the control arm will be advised strict egg avoidance including all baked egg products. However for those who were already previously tolerating baked egg products, they will be allowed to continue consumption of baked egg products outside of study, up to a maximum of 2.5g baked egg protein (approximately equivalent to a slice of cake or one muffin) per week.

Subjects in the BER group undergoing cookie OIT will be counselled for strict egg avoidance including all baked egg products outside of the study treatment. Subjects in the BET group who had tolerated baked egg products prior to study will be allowed to continue the consumption of baked egg products outside of study, up to a maximum of 2.5g baked egg protein (approximately equivalent to a slice of cake or one muffin) per week. This limit is placed because baked egg consumption in BET subjects may enhance desensitisation to cooked egg, and subjects taking larger quantities of baked egg outside of pancake OIT may have better rates of desensitisation. However, as pancake OIT outcomes should be assessed

in a real-world setting, this will include allowing baked egg consumption in egg allergic children who are already tolerating baked egg.

Subjects who are receiving anti-IgE therapy, oral immunosuppressants, beta-blocker, ACE inhibitor or immunotherapy for other allergens will fall under the study's exclusion criteria and be withdrawn from study.

Subjects will be advised to avoid intense physical exercise up to 2 hours before and after each OIT dose intake.

6.5 Blinding

This is an open label study. Blinding will not be performed.

6.6 Concomitant therapy

Participants may continue usual medications, including those taken for asthma, rhinitis, and eczema during the study. Antihistamines must not be taken on the day of updosing and must be stopped prior to FC visits as per Section 4.3.2.8. Participants (or their parents) will be asked to document any use of medication (including rescue medication) in home diary cards.
All medications (prescription and over the counter) taken by the participant should be documented.

7 SAFETY MEASUREMENTS

7.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.
A serious adverse event (SAE) or reaction is any untoward medical occurrence that at any dose:

- results in or contributes to death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in or contributes to persistent or significant disability/incapacity or
- results in or contributes to a congenital anomaly/birth defect
- results in such other events as may be prescribed

Important Notes Relating to Definition of SAE:

- Hospitalisation refers to the requirement for overnight admission to hospital. Observation in hospital following an allergic reaction that is not immediately life threatening does not constitute a hospital admission.

- A subject who experiences an anaphylaxis reaction (e.g. hoarse voice, cough, wheeze, stridor not requiring intubation, transient hypotension) is not at "immediate risk of death" and such events will not be classified as SAE unless they fulfil SAE criteria.

The following criteria will constitute an SAE:

- Anaphylaxis occurring at in-hospital challenge *requiring more than two doses* of intramuscular adrenaline
- Anaphylaxis occurring in response to a dose of OIT outside the hospital environment, *requiring more than one dose* of intramuscular adrenaline.
- Clinician-confirmed diagnosis of eosinophilic oesophagitis (with positive histology).

7.2 Causality

The adverse event can be described as **'expected'** if it caused symptoms and/or signs that could be reasonably described as a consequence of an allergic reaction to allergen exposure within the protocol. Symptoms of an allergic reaction are defined as any described within this protocol. An adverse event is considered **"unexpected"** when its nature or severity is not consistent with the investigator's protocol.

An adverse event is defined as **"related"** when it has resulted from the administration of any of the research procedures. Related adverse events are defined as **"adverse reactions".** All episodes of anaphylaxis occurring in response to a dose of OIT will be treated as an **AE "of interest"**.

In this study, the following may be used as a guide to aid consistency in classification of the relationship to study treatment. The descriptors outlined below are not intended to be prescriptive and the Site Investigator should classify each event according to the specific circumstances involved.

Probably related:

- Objective signs of an IgE mediated allergic reaction (hives, angioedema, vomiting, anaphylaxis) occurring within 1 hour of the study treatment in the absence of other known allergic triggers for that subject; AND
- Symptoms **cannot** be reasonably explained by subject's clinical state, environmental or toxic factors or other treatments taken by the participant.

Possibly related:

- Objective signs of an IgE mediated allergic reaction (hives, angioedema, vomiting, anaphylaxis) occurring more than 1 hour but less than 4 hours after taking the study treatment; AND
- Symptoms **cannot** be reasonably explained by subject's clinical state, environmental toxic factors or other treatments taken by the participant.

Unlikely to be related:

- Allergic symptoms occurring more than 4 hours after taking study treatment; or
- Symptoms **can** be reasonably explained by the subjects' clinical state, environmental or toxic factor or other therapies taken by the participant. e.g. exacerbation of asthma or eczema; or
- Symptoms do not follow a known pattern of response for the study treatment.

Unrelated:

- Any event where an alternate cause is identified – e.g. allergic reaction following exposure to another food allergen in a subject with known allergy to that allergen; or
- The AE does not meet the criteria for the other categories above.

Not assessable:

- There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

7.3 Collecting, Recording and Reporting of Adverse Events and Serious Adverse Events to CIRB

All adverse events will be recorded on a subject's diary 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. All adverse events will be recorded, regardless of their severity or relation to study medication or procedures.

Reporting of adverse events involves the PI submitting to the approving CIRB the completed SAE Reporting Form within the stipulated timeframe. PI is responsible for informing the institution representative (local SAE resulting in death), sponsor or regulatory bodies as required and appropriate.

Reporting timeline to CIRB:

- SAE that result in death, regardless of causality, should be reported immediately - within 24 hours of the PI becoming aware of the event.
- All "related" SAE (definitely / probably / possibly) irrespective of severity or expectedness, need to be reported to CIRB within 24 hours of the PI becoming aware of the event.

7.4 Grading of Adverse Events

Allergic reactions will be graded according to the CoFAR Grading System for Allergic Reactions, with additional modification to Grade 1 symptoms as shown in Table 8.

Grade 1 - Transient	Grade 1 – Mild	Grade 2 – Moderate	Grade 3 – Severe	Grade 4 – Life Threatening	Grade 5 Death
Transient mild discomfort (<20mins). No limitation in activity	Mild discomfort (< 48 hours). No limitation in activity	Symptoms produce mild to moderate limitation in activity.	Marked limitation in activity.	Extreme limitation in activity.	Death
No assistance or medical intervention / therapy required.	No assistance or medical intervention/ therapy required.	Some assistance may be needed; no or minimal intervention/ therapy is required. Hospitalization is possible but unlikely.	Assistance usually required; Medical intervention is required (Parenteral medication(s) are usually indicated) Hospitalisation is possible.	Significant assistance and medical intervention is required; hospitalisation is probable.	
Symptoms may include pruritus, periorbital or facial angioedema, swelling or rash, mild abdominal discomfort, or other transient symptoms.		Symptoms may include persistent hives, wheezing without dyspnea, abdominal discomfort / increased vomiting, or other symptoms	Symptoms may include bronchospasm with dyspnoea, severe abdominal pain, throat tightness with hoarseness, transient hypotension among others.	Symptoms may include persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/ or incontinence or other life-threatening symptoms.	

Table 8: CoFAR Specific Grading System for Allergic Reactions

7.5 Safety Monitoring Plan

The PI will perform regular data and safety monitoring. Safety data including side effects and adverse reactions will be monitored from time of enrolment to completion of study. The PI will report AE/ SAE to CIRB as per guidelines stipulated above.

7.6 Complaint Handling

Complaints will be handled by the PI and dealt with according to the institution's guidelines.

8 DATA ANALYSIS

8.1 Data Quality Assurance

The PI and co-I (at least 2 persons) will independently and regularly check each other's data collection forms, data entry, to ensure accurate and complete data collection and data entry, in accordance to the protocol. Any disagreements will be discussed together with the third co-I for consensus.

8.2 Data Entry and Storage

Data will be first entered into hardcopy data collection forms (DCF). These forms will be stored under lock and key in access-controlled Paediatric office, only accessible to the study team. Data will then be transcribed from DCF to electronic forms (Microsoft Excel/ SPSS). Coded data will be stored on password-protected files on password-protected computers' shared folders, and all identifying information will only be available to the researchers in a file separate from the coded data that is also password-protected.

9 SAMPLE SIZE AND STATISTICAL METHODS

9.1 Determination of Sample Size

The incidence of natural resolution of egg allergy over 12 month period is about 15% (4),(10). The efficacy for desensitisation using raw/cooked eggs is about 80% (ranging between 55% -100%) whilst that for OIT using baked egg products is about 50%. As there are no prior studies using pancake to desensitise egg-allergic patients, we are estimating the efficacy to be in between that of raw/cooked eggs and baked eggs, at about 65%. We plan to recruit 33 egg-allergic children to the study, who will be randomised 2:1 to active (n=22) or control (n=11) groups. This will allow us to detect a difference in rates of desensitisation of 50%, with 80% power using a two-sided significance level of 0.05, whilst allowing for a 30% attrition rate. Sample size calculation was performed using PASS 14 (Power Analysis and Sample Size Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.).

9.2 Statistical and Analytical Plans

The primary analysis will be performed on an intention to treat basis: all subjects allocated to each arm will be analysed together as representing that treatment arm, irrespective of compliance and whether or not they have completed the prescribed regimen. A secondary

analysis will also be performed on a per protocol basis. Statistical analysis was performed using SAS software version 9.4 for Windows (Cary, NC: SAS Institute Inc.). Statistical significant is set at $p < 0.05$.

9.2.1 Analysis of Primary Outcome

The primary analysis will compare the proportion of subjects who can tolerate 4443mg or more of egg protein in the active and control groups, using a two-sided Fisher's exact test.

9.2.2 Analysis of Secondary Outcomes

The following secondary analyses will be performed:

- Proportion of subjects who tolerate 4443mg or more of egg protein in the active and control groups, following cessation of all egg intake for 6 weeks (sustained unresponsiveness), using a two-sided Fisher's exact test.

- The incidence of adverse events experienced between active and control groups. Statistical differences will be determined using two-sided Fisher’s exact test for proportions; non parametric tests for graded reactions. Logistic regression will be used to assess the association of age, gender, asthma status and prior history of anaphylaxis with incidence of adverse events.

- Completion rates in the active and control groups will be compared using a two---sided Fisher's exact test.

- Change in quality of life assessments before and after OIT, as assessed by FAQL-Q in children, teenagers and their parents – between the active and control groups using two independent sample t test (or Mann-Whitney U test if normality assumption is not tenable). The changes within each group will be assessed using paired t test (or Wilcoxon signed rank test if normality assumption is not tenable).

- Immunological outcome measures pre- and post- of OIT, using paired t test (or Wilcoxon signed rank test if normality assumption is not tenable) within each group.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigators will permit study-related monitoring, audits and/or IRB review and regulatory inspection(s), providing direct access to source data/document.

11 QUALITY CONTROL AND QUALITY ASSURANCE

The PI and co-I (at least 2 persons) will independently and regularly check each other's data collection forms, data entry, to ensure accurate and complete data collection and data entry, in accordance to the protocol. Any disagreements will be discussed together with the third co-I for consensus.

12 ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the Good Clinical Practice and the applicable regulatory requirements.

This final study protocol, including the final version of the Patient Information and Informed Consent Form, must be approved in writing by the Centralised Institutional Review Board (CIRB), prior to enrolment of any patient into the study.

The principle investigator is responsible for informing the CIRB of any amendments to the protocol or other study-related documents, as per local requirement.

12.1 Informed Consent

Prior to study enrolment, written informed consent will be obtained from at least one of the parents, or legal representative of the participating subject. The PI/ co-I will obtain this written informed consent after adequate explanation to the parents/ legal representative, of the aims, methods, anticipated benefits and potential risks of the study and the discomfort it may entail. Written informed consent will be obtained prior to screening, with the understanding that consent may be withdrawn at any time without prejudice. Child assent forms will be signed for children above 6 years of age. Two copies of the informed consent and assent will be signed: one set is given to the subject’s parents, and one set is retained on site by the investigator. In obtaining and documenting informed consent, the investigators will comply with the GCP guidelines and to the ethical principles that have their origin in the Declaration of Helsinki.

12.2 Confidentiality of Data and Patient Records

Coded data will be stored on password-protected files on password-protected electronic storage devices (thumbdrives), and all identifying information will only be available to the researchers in a file separate from the coded data that is also password-protected. The password-protected thumbdrives will be stored under lock and key in access-controlled

Paediatric office. Primary research data will only be available to the entire study team; the analysed or tabulated results without identifying features would be made available to the institution as required.

13 PUBLICATIONS

Study findings will be submitted to a peer-review journal for publication and authorship will be accorded based on contributions.

14 RETENTION OF TRIAL DOCUMENTS

Records for all participants, including data collection forms, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation should be retained by the PI in a secure storage facility. The records should be accessible for inspection and copying by authorized authorities. The records will be retained and stored for a minimum of 7 years after completion of research study or date of publication of the research using the research data, whichever is later.

15 FUNDING and INSURANCE

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