Improving the safety of oral immunotherapy for cow's milk allergy

(The SOCMA Study)

Version 2.1 dated 11 December 2018

SPONSOR: Imperial College London

FUNDING: J.P. Moulton Charitable Foundation
Sociedad Española de Alergología e Inmunología Clínica (SEAIC)
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica (SEICAP)

STUDY CENTRES: Imperial College London, UK & Niño Jesús Hospital, Madrid, Spain

NRES reference: 18/L0/1070

Protocol authorised by:

Name & Role	Date	Signature

AMENDMENT HISTORY

Amendment No.	Protocol Version	Date issued	Author(s) of changes	Details of Changes made
1	2.1	11 Dec 2018	PAUL TURNER (CI)	1. Change in maximum dose in Phase 1 updosing from 70mg to 60mg. Correspondingly, 70mg dose in Phase 2 updosing altered to 60mg. 2. Removal of basophil activation test (under mechanistic assessments) 3. Addition of Dr Carmelo Escudero as co-PI in Madrid centre 4. Minor typos corrected

PROTOCOL SYNOPSIS

Title	Improving the Safety of Oral immunotherapy for Cow's Milk Allergy
Abbreviated title	SOCMA study
Clinical Trials.gov number	NCT02216175
IRAS Number	174513
Sponsor R&D Number	18SM4569
HRA Ethics reference	18/L0/1070
Primary objectives	To evaluate the impact of a sublingual immunotherapy (SLIT) pretreatment on the safety of oral immunotherapy (OIT) in children with persistent IgE-mediated cow's milk (CM) allergy.
Secondary objectives	1. To evaluate the safety and efficacy of CM-SLIT in children with IgE-mediated CM allergy. 2. To assess whether the impact of SLIT pretreatment is dependent on sublingual exposure, or whether the same effect can be achieved through very low dose OIT. 3. To evaluate predictors which can identify individuals likely to undergo successful desensitization. 4. To assess the impact of CM immunotherapy on health-related quality of life.
Intervention	A two centre, parallel group, three arm, randomised placebo-controlled trial, comparing the safety and efficacy of OIT, with and without pretreatment with sublingual immunotherapy. At the end of each phase, efficacy of treatment will be assessed by double-blind, placebo-controlled food challenge (DBPCFC), to determine the change in threshold of reactivity for each participant.
Safety	Study visits will take place in a dedicated hospital paediatric research unit, by personnel qualified in the recognition and treatment of anaphylaxis, and observed for at least 60 minutes following a dose. All families will be provided with an Allergy Management Plan, prescription for rescue medication including adrenaline auto-injector devices, and appropriate training in the recognition and management of allergic reactions.
Patient group	Children and young people with a diagnosis of IgE-mediated cow's milk allergy between 6 - 17 years old. Target recruitment of 66 subjects
Sponsor	Imperial College London
Funding	J.P. Moulton Charity Foundation Sociedad Española de Alergología e Inmunología Clínica (SEAIC) Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica (SEICAP)

Study Investigators

Joint Chief Investigators: Dr Paul Turner, Imperial College London
Dr Marta Vazquez-Ortiz, Imperial College Healthcare NHS Trust, London

Co-investigators: Dr Pablo Rodríguez del Río, Niño Jesús Hospital, Madrid, Spain
Dr Silvia Sanchez-Garcia, Niño Jesús Hospital, Madrid, Spain
Dr Carmelo Escudero, Niño Jesús Hospital, Madrid, Spain
Dr Maria Dolores Ibañez Sandin, Niño Jesús Hospital, Madrid, Spain

Dr Mohamed Shamji, Imperial College London
Dr Belén de la Hoz Caballer, Hospital Universitario Ramón y Cajal, Madrid, Spain
Dr Javier Martinez-Botas, Hospital Universitario Ramón y Cajal, Madrid, Spain

Study sites

1. Imperial College London / Imperial College Healthcare NHS Trust
   Site PIs: Dr Paul Turner & Dr Marta Vazquez-Ortiz
2. Niño Jesús Hospital, Madrid, Spain
   Site PIs: Dr Pablo Rodríguez del Río, & Dr Silvia Sanchez-Garcia

Study Coordination Centre

For general queries, supply of trial documentation, and collection of data, please contact:

Study Coordinator: Dr Paul Turner
Address: Paediatric Clinical Research Facility,
2nd Floor Cambridge Wing, St Mary's Hospital, Praed Street, London W2 1NY
Telephone: 020 3312 7754 **Fax**: 020 3312 7571
Email: p.turner@imperial.ac.uk

Study Management Group

Joint Chief Investigators: Dr Paul Turner, Imperial College London
Dr Marta Vazquez-Ortiz, Imperial College Healthcare NHS Trust, London

Co-investigators: Dr Pablo Rodríguez del Río, Niño Jesús Hospital, Madrid, Spain

Trial Steering Committee

Independent Chair: Dr Gary Stiefel (UK)
Independent Members: Dr Alberto Alvarez (Spain)
Ms Karen Brunas (UK)

Chief Investigator: Dr Paul Turner (UK)
Co-Investigator: Dr Pablo Rodríguez del Río (Spain)

Independent Data Monitoring Committee

Chair: Prof Jurgen Shwarze (UK)
Members: Dr Joan Bartra (Spain)
Ms Sarah Lindsley (UK)

Clinical Queries

Clinical queries should be directed to Dr Paul Turner who will direct the query to the appropriate person

Sponsor

Imperial College London is the research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 221
Medical School Building
St Marys Campus
Norfolk Place
London W2 1PG

Funding

J.P. Moulton Charity Foundation (Clinical study, London)
Sociedad Española de Alergología e Inmunología Clínica (SEAIC) (Clinical study, Madrid; mechanistic work)
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica (SEICAP) (mechanistic work)

This protocol describes the SOCMA study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	TERM
AE	Adverse Event
BSACI	British Society for Allergy & Clinical Immunology
CM	Cow's milk
DBPCFC	Double blind, placebo-controlled food challenge
FAQL-Q	Food Allergy Quality of Life - Questionnaire
IDMC	Independent Data Monitoring Committee
LOAEL	Lowest observed adverse event level
NOAEL	No observed adverse event level
OIT	Oral Immunotherapy
QoL	Quality of Life
SAE	Serious adverse event
SAR	Serious adverse reaction
SCORAD	SCORing Atopic Dermatitis tool
SLIT	Sublingual immunotherapy
SPT	Skin Prick test

TABLE OF CONTENTS

1. INTRODUCTION

1.1 BACKGROUND

Cow's milk allergy is the most common food allergy in young children, affecting up to 1 in 30 infants.[1] Allergic reactions are unpredictable, and have a major impact on the quality of life for the child and their family due to the potential for life-threatening allergic reactions (anaphylaxis) and the social/dietary restrictions needed to mitigate this risk.

There is no routine treatment available for CM allergy in the UK: day-to-day management involves avoidance of dairy foods and the availability of rescue medication (such as an adrenaline 'pen') in the event of an accidental reaction. Milk is ubiquitous in our diets, so avoidance is very difficult, especially as children grow older. In one study, CM was detected in 43% of the bakery products, with 21% containing sufficient CM to cause an allergic reaction in 10% of children with CM allergy.[2] Unsurprisingly, 2 in 5 children with CM allergy will have allergic reactions due to accidental reaction *every year*. While some will outgrow their milk allergy by school age, recent studies have shown that up to 50% will continue to remain allergic into their teens and beyond,[1] and this group are at greater risk of severe reactions and death. **Dietary avoidance is not a treatment – it is a management strategy**. Our aim is to develop a safe treatment for these young people.

One approach, used in some specialist centres in continental Europe and USA, is immunotherapy. Oral immunotherapy (OIT) involves administering very small but increasing amounts of CM daily for several months, and is effective for milk allergy, but causes significant reactions in some people. Milder reactions occur in 6 to 30% of doses, particularly in those with persisting CM allergy[3]. **We wish to test a novel approach to improving CM-OIT safety, to a level acceptable for standard clinical practice.**

Sublingual immunotherapy (SLIT), where the dose of CM is kept under the tongue for a few minutes, prior to swallowing, has a favourable safety profile. SLIT has revolutionised the field of immunotherapy to treat hay fever, particularly in children, allowing many more patients to access the treatment than previously due to improved safety and ease of updosing. Two studies have assessed SLIT for CM allergy,[4] with a reassuring safety profile. **However, clinical efficacy was much more limited than for OIT – a major limitation of SLIT done in isolation.**

We propose to use SLIT pretreatment, at a higher dose than that previously used, followed by a standard OIT, to improve the safety and efficacy of OIT for CM allergy.[5] Proof-of concept data exists relating to the use of SLIT pretreatment prior to peanut OIT in 9 children: the combination led to over 70% reduction in relative risk of systemic allergic reactions.[6] A similar, well-powered study for CM allergy is thus warranted.

[1]Turner PJ. Pediatr Allergy Immunol 2013; 24:624-6.
[2]Trendelenburg et al. Allergy. 2015 May;70(5):591-7.
[3]Vázquez-Ortiz M, Alvaro-Lozano M, Alsina L, et al. Clin Exp Allergy 2013; 43:92-102.
[4]Keet et al. J Allergy Clin Immunol. 2012; 129:448-55, 455.e1-5. De Boissieu et al. Allergy 2006; 61:1238-9.
[5]Vazquez-Ortiz & Turner. Pediatr Allergy Immunol. 2016;27(2):117-25.
[6]Narisety et al. J Allergy Clin Immunol. 2015; 135:1275-82.e1-6.

1.2 STUDY RATIONALE & JUSTIFICATION

We wish to formally assess, in a randomised, placebo-controlled study, whether SLIT pretreatment prior to OIT can improve the safety of oral desensitisation in children with persisting CM allergy. We will also evaluate whether the effect of SLIT is dependent on exposure via the sublingual route, or can be achieved when the equivalent dose is given orally (as very low dose OIT). We will assess the safety and efficacy of these approaches, and study the immunological mechanisms involved, our secondary aim being to develop clinically-useful predictors for identifying individuals likely to undergo successful desensitization.

STUDY HYPOTHESES:

1. Pretreatment with sublingual immunotherapy (SLIT) improves the safety of subsequent oral Immunotherapy (OIT) in children with cow´s milk allergy.

2. The effect of pretreatment is dependent on exposure via the sublingual route: the same benefit is not obtained if pretreatment is directly swallowed.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To evaluate the impact of SLIT pretreatment on the safety of OIT in children with IgE-mediated CM allergy.

2.2 SECONDARY OBJECTIVES

- To evaluate the safety and efficacy of CM-SLIT in children with IgE-mediated CM allergy.
- To assess whether the impact of SLIT pretreatment is dependent on sublingual exposure, or whether the same effect can be achieved through very low dose OIT.
- To evaluate predictors which can identify individuals likely to undergo successful desensitization.
- To assess the impact of CM immunotherapy on health-related quality of life.

3. STUDY DESIGN

The study is a two site, parallel group, three arm, randomised placebo-controlled trial, comparing the safety and efficacy of conventional OIT, with and without pretreatment with allergen immunotherapy delivered by either the sublingual or oral route (see Figure 1). At the end of each phase, efficacy of treatment will be assessed by double-blind, placebo-controlled food challenge (DBPCFC), to determine the change in threshold of reactivity for each participant. The study consists of 2 phases:

Phase 1 (duration 4-7 months): Participants will be randomised in a double-blind manner to intervention (active SLIT or low dose OIT or placebo) for 4-7 months, aiming for a 4 month period at maintenance dose.

Phase 2 (6 months duration): Open label intervention to assess the impact of the phase 1 intervention on subsequent safety of convention OIT to cow's milk.

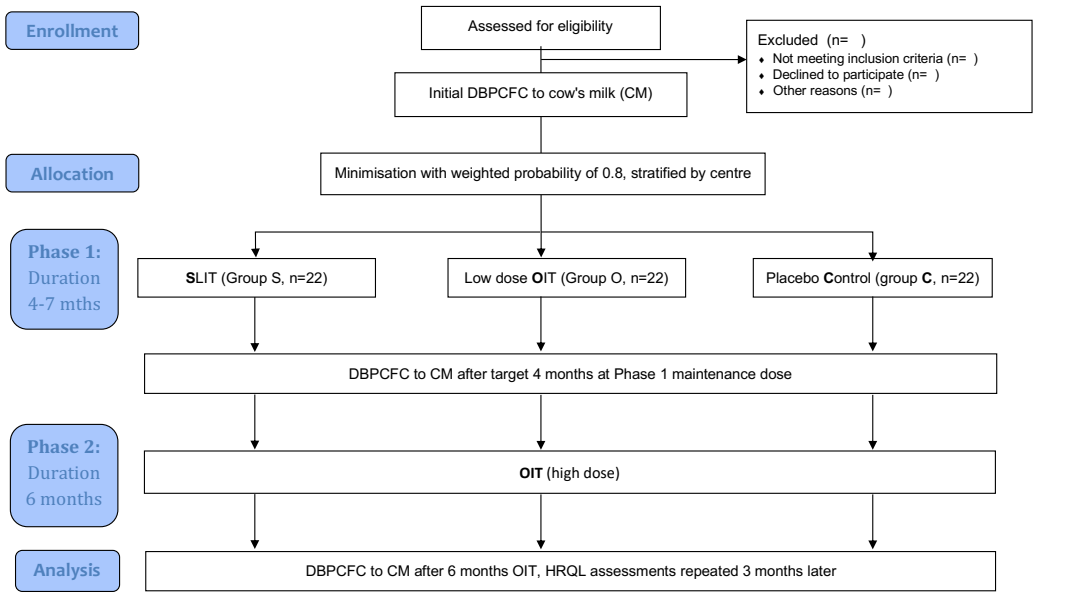


Figure 1: CONSORT diagram for the SOCMA study.
[DBPCFC: double-blind, placebo-controlled food challenge; OIT: oral immunotherapy; SLIT: sublingual immunotherapy]

Summary of study components:

1. **Recruitment and eligibility screen**: CM-allergic subjects will be recruited from both our local patient population and through local paediatric allergy networks in London and Madrid.
2. **Baseline double-blind, placebo-controlled food challenge** (DBPCFC) to CM. This will confirm clinical reactivity to CM rather than sensitisation without clinical allergy, as it is unethical to undertake CM immunotherapy in children who are not clinically allergic to CM.
3. **Treatment Allocation and Phase 1:** CM-allergic children will be randomized 1:1:1 (using minimisation with weighted randomisation) to one of the following 3 arms, for 4-7 months (due to a target of 4 months duration at maintenance dose):

i. Group S: SLIT, at a higher dose than that reported in previous studies [n=22]

ii. Group O: "low dose OIT" using the same doses as SLIT but administered orally [n=22]

iii. Group C: placebo control [n=22], half via the sublingual route, the remainder orally.

4. **Post phase 1 assessment:** All subjects will undergo a DBPCFC to CM to determine any change in threshold to CM following the Phase 1 intervention.
5. **Phase 2:** All subjects will be offered OIT using a conventional protocol for 6 months.
6. **Post phase 2 assessment:** All subjects will undergo an exit DBPCFC to CM to determine any change in threshold to CM. Blinding as to the phase 1 intervention will be preserved until after completion.

All participants will be advised on current best practice for CM allergy, i.e. CM avoidance (except for study doses) plus provision of rescue medication and training) for the duration of the study.

The impact of CM immunotherapy on HRQL will be assessed prior to, and after each phase in all subjects (and their parents), using validated questionnaires, with the final assessment 3 months after completing Phase 2.

3.1 STUDY OUTCOMES MEASURES

3.1.1 PRIMARY STUDY OUTCOME

The proportion of participants experiencing adverse events classified as mild non-transient symptoms or more severe (see table 9) during CM-OIT (phase 2), in those who have received SLIT pretreatment (group S) compared to placebo (group C).

3.1.2. SECONDARY STUDY OUTCOMES

- Proportion of participants experiencing adverse events classified as mild non-transient symptoms or more severe (see table 9) during CM-OIT (phase 2), in those who have received SLIT pretreatment (group S) compared to the equivalent SLIT dose orally during phase 1 (group O).
- Other safety outcomes in the different treatment groups (withdrawals due to intervention, anaphylaxis rate/adrenaline use)
- Efficacy defined at DBPCFC as the proportion of study participants experiencing:
  i. No symptoms (or only mild transient symptoms as per table 9) to 8 grams CM protein (approx. 250mls fresh milk) ("Complete desensitisation")
  ii. No symptoms (or only mild transient symptoms as per table 9) to at least 1.4 grams CM protein (approx. 45mls fresh milk) ("Partial desensitisation")
  iii. At least a 10-fold increase in eliciting dose (defined as the lowest dose which elicits objective symptoms or signs at challenge).

  Efficacy at 6 and 12 months will be compared across all treatment groups (including SLIT pretreatment) vs the equivalent dose administered orally without a sublingual phase (to address hypothesis 2).
- Immunological outcomes (titrated skin prick test, serum IgE) at baseline, 6 and 12 months.
- Change in health-related quality of life (HRQL) measures at 6, 12 and 15 months, as assessed by validated questionnaires (FAQLQ, FAIM, EQ-5D, self-efficacy) in study participants and their parents.

3.2 STUDY DESIGN RATIONALE

We have chosen to recruit subjects age 6+ years, as there is a lower likelihood of natural resolution after this age, and safety monitoring is easier given to the ability of children of this age to communicate symptoms more clearly. Furthermore, in our experience, children of this age (and above) are more likely to understand the commitments involved in a study of this nature.

In Phase 1, children will be allocated to receive either SLIT or placebo. Placebo reactions are common in food immunotherapy, and this has not been assessed in previous CM-SLIT studies. It is therefore important to include a placebo control arm, to allow for an assessment of safety as well as efficacy. In addition, the inclusion of a placebo arm will allow us to assess for natural resolution, where a child might outgrow their CM allergy during this period, independent of any active intervention. The duration of Phase 1 will depend on each participant's initial sensitivity to CM, in order to achieve a degree of consistency in terms of duration that each participant is receiving the highest maintenance dose (target 4 months).

Data suggests that allergen exposure across the sublingual mucosa may be more likely to induce tolerance, as the sublingual region has the highest permeability within the oral mucosa, and a higher density of dendritic cells which can stimulate T-regulatory response;[7] this might explain the efficacy of SLIT in the treatment of allergic rhinitis (hay fever). We have included a third "low dose OIT" arm in Phase 1, where the same dose of SLIT is immediately swallowed and then rinsed away (instead of being held under the tongue), as this will allow us to assess the effectiveness of the sublingual route.

Phase 2 involves all study participants undergoing 6 months of conventional OIT, thus allowing the effect of the Phase 1 treatment to be assessed. We have not included a placebo arm in Phase 2 because:

i. Our public/patient involvement (PPI) panel felt that the inclusion of a placebo control group throughout both study phases was less acceptable and would adversely affect recruitment (since some children would undergo 3 DBPCFC but no active treatment).

ii. There is existing data from placebo-controlled studies on the safety of conventional OIT for CM allergy; this information is not required for our primary outcome.

iii. The protocol already allows for an assessment for natural resolution of CM allergy, given the inclusion of a control group in Phase 1.

We will use an OIT protocol in Phase 2 that has been adapted from published and unpublished data relating to eliciting doses in children with CM allergy, as well as previous OIT protocols with favourable safety data. All updosings will take place under medical supervision. We therefore expect this protocol to have a satisfactory safety profile, even for those participants randomised to the placebo group in Phase 1.

The efficacy of treatment will be assessed by double-blind, placebo-controlled food challenge (DBPCFC) at the end of each phase, an established research tool in food allergy.[8] We have chosen a daily maintenance dose of 4000mg CM protein (roughly equivalent to 120 ml or half a cup of milk), on the basis of previous reports that this amount is generally well-tolerated by subjects undergoing OIT on a daily basis, and it is also associated with a suggestion of efficacy. Our main efficacy outcome – "successful desensitization" or ability to tolerate 8000mg CM protein – has been chosen in agreement with previous studies to allow the intake of full milk servings following the intervention. This is expected to allow dietary liberalisation, potentially leading to a positive impact on health-related quality of life.

This is a two centre study: the procedures involved are complex and require intensive clinical assessment only available at specialist allergy centres with experience in food immunotherapy. The study design allows for a more efficient use of resources and promotes consistency in clinical management. The locations (in central London and Madrid) allow for the recruitment of patients from diverse geographical areas and ethnic background.

---

[7] Jay DC, Nadeau KC. Curr Allergy Asthma Rep. 2014; 14:473.

[8] Plaut M, Sawyer RT, Fenton MJ. Summary of the 2008 NIAID–US FDA Workshop on Food Allergy Clinical Trial Design. J Allergy Clin Immunol 2009;124:671-8.e1.

4. STUDY POPULATION

4.1. INCLUSION CRITERIA

1. Age 6-17 years at time of consent.
2. Past history consistent with IgE-mediated allergy to CM.
3. Allergic to 1.44g CM protein (approx. 40ml fresh milk) or less, at DBPCFC prior to randomisation
4. Written, informed consent of parent/legal guardian and patient assent.

4.2. EXCLUSION CRITERIA

1. Required previous admission to an intensive care unit for management of an allergic reaction.
2. Significant symptoms of non-IgE-mediated CM allergy within the previous 12 months.
3. Children with a past history of CM allergy currently consuming CM-containing products other than extensively-heated milk in baked foods (e.g. biscuits, cakes).
4. Poorly controlled asthma within the previous 3 months (as defined by clinician judgement with reference to the ICON consensus)[9], or asthma requiring treatment with >5 days oral corticosteroids within the previous 3 months.
5. Moderate-severe eczema, defined as requiring *more than* once daily application of 1% hydrocortisone as maintenance treatment despite appropriate use of emollients (eczema is not otherwise an exclusion criteria)
6. Clinically significant chronic illness (other than asthma, rhinitis or eczema)
7. History of symptoms of eosinophilic oesophagitis, irrespective of cause
8. Undergoing specific immunotherapy to another allergen *and* within the first year of treatment.
9. Receiving anti-IgE therapy, oral immunosuppressants, beta-blocker or ACE inhibitor.
10. Pregnancy
11. Unwilling or unable to fulfil study requirements

4.3. WITHDRAWAL CRITERIA

Subjects will be free to withdraw from the study at any time without affecting their future medical care. Subjects who are withdrawn from active treatment will be invited to remain in the study for assessment of outcome relating to safety and HRQL measures (HRQL will be assessed 3 months after withdrawal).

The safety of study participants is paramount in this study: subjects who are assessed as having had anaphylaxis to a home dose but who do not receive 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 will 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 without due reason.

Any subject excluded from further participation will not be replaced, unless withdrawal occurs after initial DBPCFC but prior to allocation to treatment.

---

[9]Papadopoulos NG, Arakawa H, Carlsen KH, et al. International consensus on (ICON) pediatric asthma. Allergy. 2012;67:976-97.

5 STUDY PROCEDURES

The study visits and procedures are summarised in Table 1 and Figure 2.

Approx month	0m		Day 1		~6m			~12m	~15m
	Screening	Baseline DBPCFC	Escalation visit	Updosing visits	Interim DBPCFC	Escalation visit	Updosing visits	Exit DBPCFC	Final HRQL assessment
General									
Informed consent/assent	X								
Medical history	X								
Allergy / diet history	X								
Directed physical exam	X	X	X	X	X	X	X	X	
Vital signs	X	X	X	X	X	X	X	X	
Asthma control questionnaire	X	(X)			(X)			(X)	
SCORAD	X								
Spirometry	X	(X)			(X)			(X)	
Peak Flow	X	X	X	X	X	X	X	X	
Adverse events		X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
Skin prick tests (SPT)	X	X[#]			X			X	
Blood test / cannulation	(X)*	(X)*			X			X	
Full blood count	(X)*	(X)*							
Urine pregnancy test	(X)								
Saliva collection	X				X			X	
Stool collection		X*							
Intervention									
Allocation			X						
SLIT/OIT			X	X		X	X		
Outcomes									
DBPCFC (2 visits)		X			X			X	
HRQL assessments	X		X		X			X	X
SPT panel (including titrated SPT)	X				X			X	
SPT to fresh CM	X	X							
Serum IgE/IgG4	X*	X*			X			X	
Other laboratory test		X[§]			X[§]			X[§]	

Table 1: Study procedures

*may be tested at either visit, depending on local centre and patient preference

[#] limited skin prick testing only during each challenge at baseline

(X) will be performed depending on clinical need / local SOP

[§]London centre only

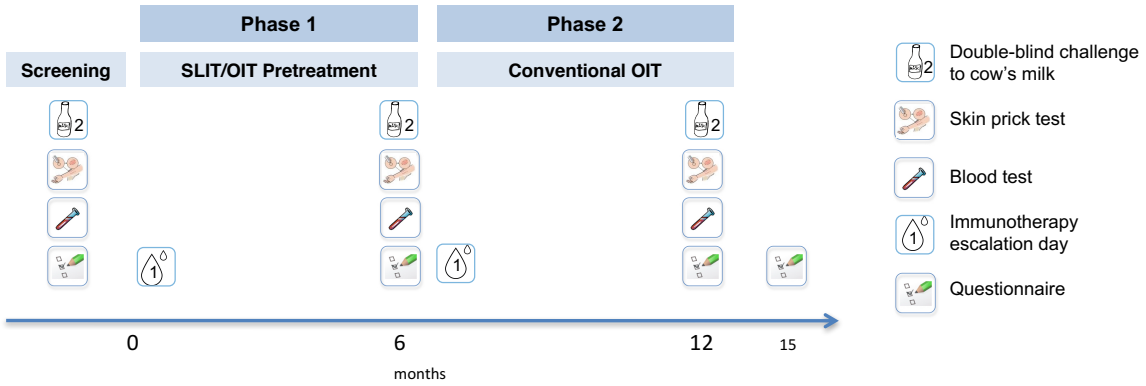


Figure 2: Study flowchart and interventions

5.1 RECRUITMENT AND SCREENING VISITS

CM-allergic children will be recruited through the clinical services at St Mary's Hospital in London (Imperial College Healthcare NHS Trust) and Niño Jesus Hospital in Madrid, two of the largest tertiary services in Europe. We will also recruit through our local paediatric allergy network in North West London. This study requires frequent visits (approximately every 10-14 days) to the Research Unit, so participants should live relatively local to the unit to minimise travel time and impact on other daily activities.

Potential participants and their families will receive the Study Information either in person, by post or via email, following which the participants will be pre-screened by telephone conversation with their parents, to determine likelihood of persistent CM allergy and thus suitability for this study. Suitable participants will then have an appointment made for a screening visit.

The following will take place at the first screening visit:

- Written informed consent and participant assent.
- Clinical history (including dietary history of CM replacement options) and physical examination
- Skin prick test (SPT) to:
    - commercial extracts of whole CM and casein (ALK-Abelló, Hørsholm, Denmark)
    - titrated SPT to fresh cow's milk (sourced from the UK), using the following dilutions (diluted with sterile saline): 1/10, 1/100, 1/1000, 1/10,000, 1/100,000.
    - SPT may also be undertaken to assess sensitisation to other foods not obviously tolerated by the participant, subject to local clinical policy
- For children with asthma, current asthma status will be assessed by the clinician with respect to the ICON consensus,[9] lung function testing and completion of a validated asthma control test
- For children with eczema, eczema will be assessed using the SCORAD system[10] (which provides an objective measure of eczema severity) as well as the POEM questionnaire, a validated patient-based symptom assessment.
- For female subjects of who have reached menarche and may be susceptible to become pregnant, a urine pregnancy test will be performed.

The screening visit may take place on the same day as the first baseline food challenge, if requested by the family, subject to local availability of staffing and facilities. Participants will also be asked to provide biological samples (blood/saliva/stool sample) at either the screening or one of the DBPCFC visits.

[10]Kunz, B., Oranje, A.P., Labreze, L., Stalder, J.F., Ring, J., and Taieb, A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. Dermatology. 1997; 195: 10–19

5.2 DOUBLE-BLIND, PLACEBO-CONTROLLED FOOD CHALLENGE TO COW'S MILK

Participants who fulfil eligibility criteria for the study will undergo a baseline double-blind, placebo-controlled food challenge to CM at enrolment, prior to randomisation. The DBPCFC is established as the gold-standard test of diagnosis of food allergy by international consensus.[11] The objectives are to:

i. confirm the diagnosis of IgE-mediated CM allergy
ii. determine the threshold of clinical reactivity to CM prior to commencing the study protocol

The DBPCFC will be repeated after each intervention phase to assess for any change in threshold.

Each DBPCFC involves 2 half-day visits, within a 28 day period, with a minimum of 72 hours in between DBPCFC visits (7 days if the first challenge results in a reaction). Participants will receive incremental doses of CM protein or placebo 20-30 minutes apart, and monitored for signs/symptoms of allergic reaction. The challenge is halted once pre-determined stopping criteria have been reached. DBPCFC will be performed according to the international PRACTALL consensus for best practice.[11]

Doses for DBPCFC will be prepared using a CM protein powder (Protifar, Nutricia Ltd, Wiltshire, UK) with equivalent casein: whey ratio to fresh milk) in a liquid matrix flavoured with Nesquik to maintain blinding. All doses used during the food challenge will be double-checked prior to administration. The order of challenge days (active or placebo control) will be randomized by an independent research associate, using a computer-generated randomization list (www.sealedenvelope.com); the randomisation key will be concealed from the study team until both pair of challenges have concluded.

5.2.1 CLINICAL ASSESSMENT AND CHECKS PRIOR TO DBPCFC

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

- No intercurrent illness (viral or otherwise)
- 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, PEFR) within the normal range.
- Baseline physical examination must not reveal any significant acute findings.

Challenges will occur on a Challenge 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 DBPCFC.

5.2.2 DBPCFC AT BASELINE AND FOLLOWING PHASE 1

The challenge will consist of up to eight incremental doses of CM/placebo given at 20-30 minute intervals, followed by an observation period of 2 hours, as shown in table 2.

Published data indicates that the eliciting dose in milk-allergic patients may be lower than for other food allergens. As a safety measure, during the baseline DBPCFC, the dosing interval between the first (0.5mg) and second (3mg) doses will be extended to 60 minutes. The additional 30 minutes will allow for the detection of symptoms in the most sensitive participants, thus limiting the chance of further but unnecessary CM doses being given.

[11]Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, et al. Standardizing double-blind, placebo-controlled oral food challenges: AAAAI-EAACI PRACTALL consensus report. J Allergy Clin Immunol. 2012;130:1260-74.

The time intervals following other doses may be extended up to one hour at the local 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).

For safety reasons, families will be asked to provide their child with a snack 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 research unit. This is to avoid the possibility of the participant experiencing further symptoms outside the hospital when eating for the first time after challenge, something which has been noted in previous studies.

Entry and 6 month DBPCFC to Cow's Milk			
Dose	CM protein (mg)	Approximate equivalent volume of fresh CM (ml)	Minimum time interval after dose
1	0.5	0.015	60 mins at baseline DBPCFC, 20-30 mins for 6m DBPCFC
2	3	0.09	20-30 mins
3	10	0.3	20-30 mins
4	30	0.9	20-30 mins
5	100	3	20-30 mins
6	300	9	20-30 mins
7	1000	30	20-30 mins
8	3000	90	2 hours observation
Cumulative dose	4444	~130	
2 hours observation period following last dose to monitor for signs of reaction			

Table 2. Dosing protocol for DBPCFC to CM.

At the second visit of the baseline DBPCFC, the local investigator may elect to give participants who fail to react at either DBPCFC visit a single dose of cow's milk to a cumulative dose of 8 grams (approximately 250ml CM) in an open unblinded manner, 2 hours after the last challenge dose, to confirm tolerance.

5.2.3 EXIT DBPCFC AT 12 MONTHS

The challenge will consist of up to nine consecutive, increasing doses of CM (or placebo) at 20-30 minute intervals, up to a cumulative dose of 8 gram CM protein, as shown in table 3.

Exit DBPCFC to Cow's Milk		
Dose	CM protein (mg)	Approx. volume of CM (ml)
1	0.5	0.015
2	3	0.09
3	10	0.3
4	30	0.9
5	100	3
6	300	9
7	1000	30
8	3000	90
9	3600	105
Cumulative dose	8044	250
2 hours observation period following last dose to monitor for signs of reaction		

Table 3. Dosing protocol for exit DBPCFC to CM.

5.2.4 ASSESSMENTS DURING FOOD CHALLENGES

The baseline food challenges are categorised as 'high-risk' on the basis that we expect the challenges to be positive. To minimize risk to the patient, all challenges will take place in a clinical environment where food challenges are performed on a regular basis, and staff are familiar with the management of allergic reactions including anaphylaxis through clinical experience and regular training.

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. In order to minimize discomfort to the child, we will offer local anaesthetic cream to be applied at least 30 minutes prior.

At both sites, blood (up to 30ml, equivalent to 1.5ml/kg) will be collected at baseline, 6 and 12 months for laboratory assessments relating to CM allergy and assessment of tolerance (see section 5.7.2). The baseline blood sample may be collected at the screening visit, or deferred to the initial DBPCFC where it can be taken from the intravenous cannula, thus reducing the number of blood tests / needle-sticks required. The blood sample will be used to provide baseline haematology (blood count) and for mechanistic investigations (as outlined in section 5.8.3).

In the London centre, additional blood samples will be collected at the initial baseline DBPCFC visits, prior to the start and during the challenge via the cannula, in order to confirm a clinical reaction using objective laboratory assessments. Since the cannula will already be sited, this will not cause discomfort to the child; where the cannula does not bleed back, no further venepuncture will be attempted. A maximum total volume of 2.5ml/kg (max 100ml) blood will be collected per visit. The blood volume is consistent with national and international guidelines on the volume of blood which can safely be taken from a child.[12]

During DBPCFC, participants will be monitored including heart rate, blood pressure and oxygen saturations. PEFR will be assessed prior to challenge, and should be repeated in the event of any respiratory symptoms or at the study team's discretion. Clinical manifestations will be assessed throughout the challenge.

Skin prick testing to fresh CM and commercial CM extract (together with positive and negative control) will be performed prior to each baseline DBPCFC. The wheal response will then be measured at approximately 30 minute intervals thereafter, until the wheal has reduced to 3mm or less, or the patient is discharged (see section 5.8.1).

5.2.4.1 ASSESSMENT OF ALLERGIC SYMPTOMS

In brief, 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,[9] as shown in Figure 3; where possible, challenges should be stopped on the basis of at least one objective symptom:

[12] Howie SRC. Blood sample volumes in child health research: review of safe limits. Bulletin of the World Health Organization 2011;89:46-53. doi: 10.2471/BLT.10.080010

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.[9]

5.2.5 MANAGEMENT OF SYMPTOMS DURING DBPCFC IN HOSPITAL

Any allergic reaction occurring during in-hospital food challenge will be managed according to established local protocol and consistent with national guidelines. The UK Resuscitation Council guideline is shown below for information only. A suggested protocol for the management of refractory anaphylaxis appears in Appendix 1.

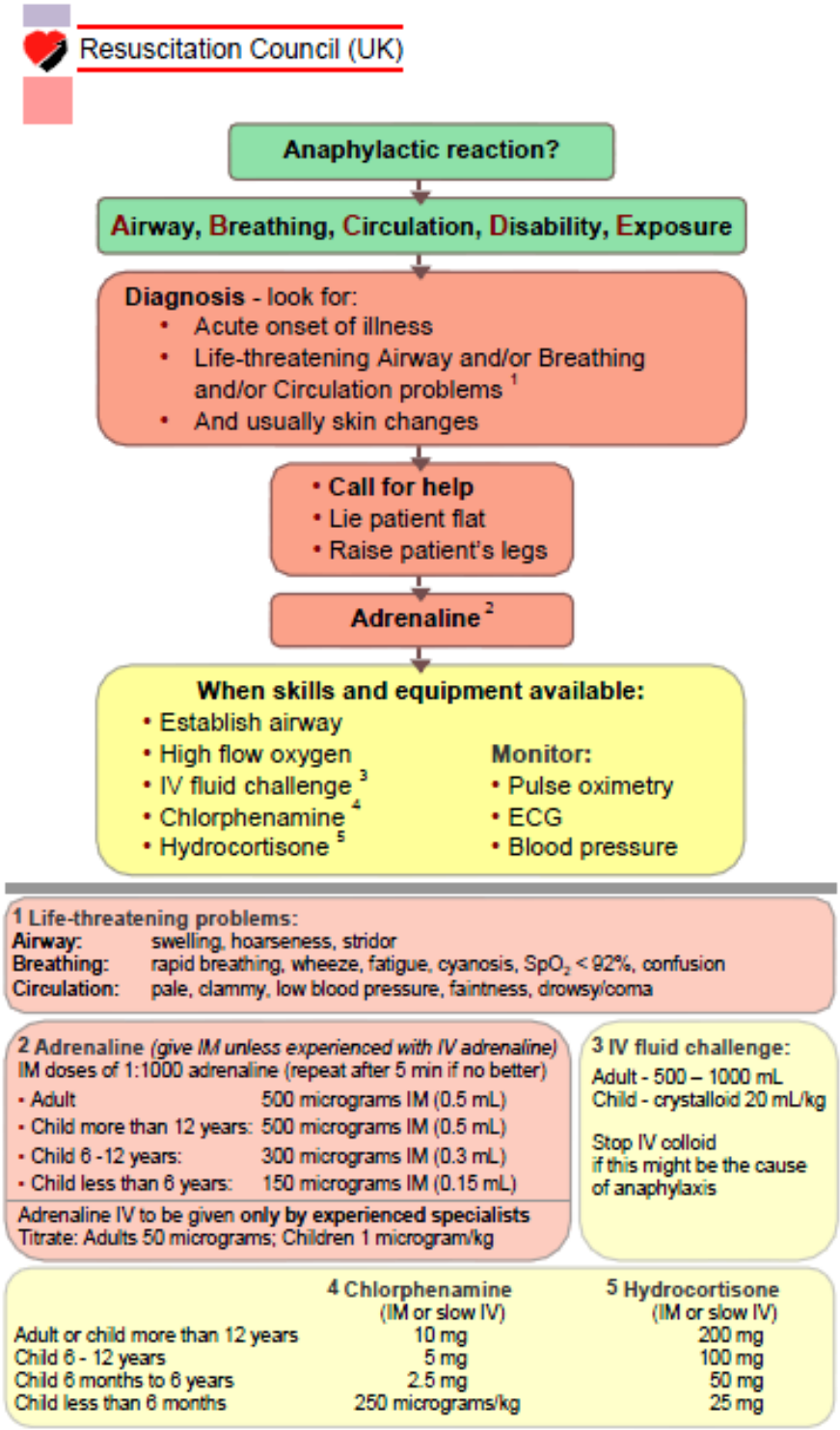


Figure 4: UK Resuscitation Council guideline for management of Anaphylaxis

5.3 TREATMENT ALLOCATION AND PHASE 1 INTERVENTION

Eligible participants (who have a positive DBPCFC, confirming IgE-mediated CM allergy) will be allocated using minimisation to one of three interventional arms, either:

- GROUP S (**S**ublingual): SLIT.
- GROUP O: Low dose **O**IT (CM protein taken orally, at the same doses used in Group S).
- GROUP C: Placebo **C**ontrol. Participants in group C will be randomized (1:1) to either placebo SLIT or placebo OIT.

5.3.1 TREATMENT ALLOCATION

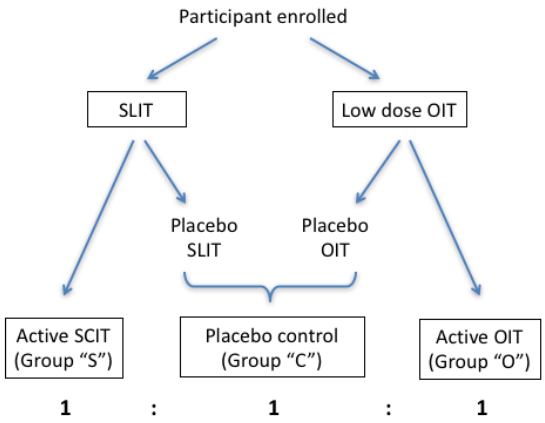
OIT outcomes are affected by a range of variables, including age, allergen-specific IgE to CM, threshold and the presence of asthma. It is therefore essential to maintain balance between treatment groups with respect to these predictors. However, stratified randomisation using several variables is not 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.[13] 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.

Participants will therefore be allocated using an online minimisation/randomization tool, Minim, with a random element using a weighting probability of 0.8, stratified by centre. Baseline variables included in the minimisation will be:

- age (under 12yrs, 13+yrs)
- sex
- specific IgE to CM (<30 kU/l, ≥30 kU/l)[3]
- eliciting dose at DBPCFC (≤44mg, 144-444mg, >444mg CM protein; these cut-offs are based on published population-based dose distributions in CM-allergic children)
- documented tolerance to CM in baked foods (tolerant or allergic/unknown).
- asthma (none, no regular inhaled corticosteroid (equivalent to BST Step 1), regular ICS (equivalent to BTS Step 2 or above).

Group allocation (active vs placebo) will be concealed throughout the study until completion of the 2nd visit of the exit DBPCFC at 12 months.

The schema for treatment allocation will be as follows:



[13] Altman DG, Bland JM. Treatment allocation by minimisation. BMJ. 2005 Apr 9; 330(7495): 843.

5.3.2. PHASE 1 (PRETREATMENT)

Following allocation, patients will commence phase 1 (pretreatment), as follows:

- Group S: Participants will receive daily SLIT for approximately 4-7 months using concentrated powdered milk with a top dose of 60mg of CM protein. Participants will be instructed to keep the SLIT dose under the tongue for 2 minutes and then swallowed ("sublingual route").
- Group O: Participants receive OIT for approximately 4-7 months using the same product and dosing protocol as that used in Group S. However, the daily dose will be administered straight into the mouth and immediately swallowed and the mouth rinsed afterwards ("oral route").
- Group C: Participants receive placebo. Participants in this group will be randomized (1:1) to either placebo SLIT or placebo OIT, following the same instructions as above.

Participants will receive daily doses of CM or placebo at home. Dose increases will be performed under medical supervision on the Research Unit, according to the updosing regime in Table 4.

5.3.2.1. INITIAL ESCALATION DAY

Phase 1 will start with an "escalation day" visit in which up to 7 consecutive doses (starting with 0.01 mg of CM protein) will be given, every 30-60 minutes (Table 4) under medical supervision. Doses will be given either by the sublingual or the oral route according to group allocation.

Participant will be assessed prior to dosing to ensure suitability, as described in section 5.2.1. If a given dose triggers symptoms which cause discomfort to the participant (such as sustained oral itch) or deemed to be significant by the research team, no further doses will be administered on the escalation day. Participants and/or their family will then be instructed to take the last well-tolerated dose on a daily basis at home, for a minimum of 10 days until the next increase is performed.

PHASE 1 UPDOSING PROTOCOL

Steps	CM protein (mg)	Volume of mix (ml)
Step 1	0.01	0.5
Step 2	0.02	0.5
Step 3	0.04	0.5
Step 4	0.08	0.5
Step 5	0.2	0.5
Step 6	0.4	0.5
Step 7	1	0.5
Step 8	2.5	0.5
Step 9	6	0.5
Step 10	15	0.5
Step 11	30	0.5
Step 12	60	0.5

Table 4: Updosing protocol for phase 1 CM immunotherapy pretreatment

Each step will be administered daily at home for a minimum of 10 days. Once the target dose is achieved (step 12), participants will continue this dose on a daily basis for a target of 16 weeks. The duration of Phase 1 will vary from participant to participant, with the aim of achieving Step 12 within 12 weeks and maintaining Step 12 for 16 weeks. Thus, the duration of Phase will vary from approximately 4 to 7 months, at which time the DBPCFC assessment will be completed and phase 2 intervention commenced.

The starting point in phase 1 (initial dose to be administered on the escalation day) will be individualised based on the outcome of the screening DBPCFC, as shown in Table 5. The NOAEL (No Observed Adverse Event Level or highest dose *not* triggering symptoms) will be used for this purpose, defined as the lowest dose tolerated at DBPCFC excluding transient Grade 1 symptoms (see Table 9).

Tolerated dose (NOAEL) at baseline DBPCFC	**Initial dose for Phase 1**		**Max dose at Initial Escalation day**		**Max number of doses at initial escalation**
	Step	**CM Protein (mg)**	**Step**	**CM Protein (mg)**	
<0.5mg (i.e. symptoms at first dose during DBPCFC)	1	0.01	7	1	7
0.5mg	5	0.2	8	2.5	4
3mg	6	0.4	9	6	4
10mg	8	2.5	10	15	3
30mg	9	6	11	30	3
100mg	10	15	12	60	3
300mg	11	30	12	60	2

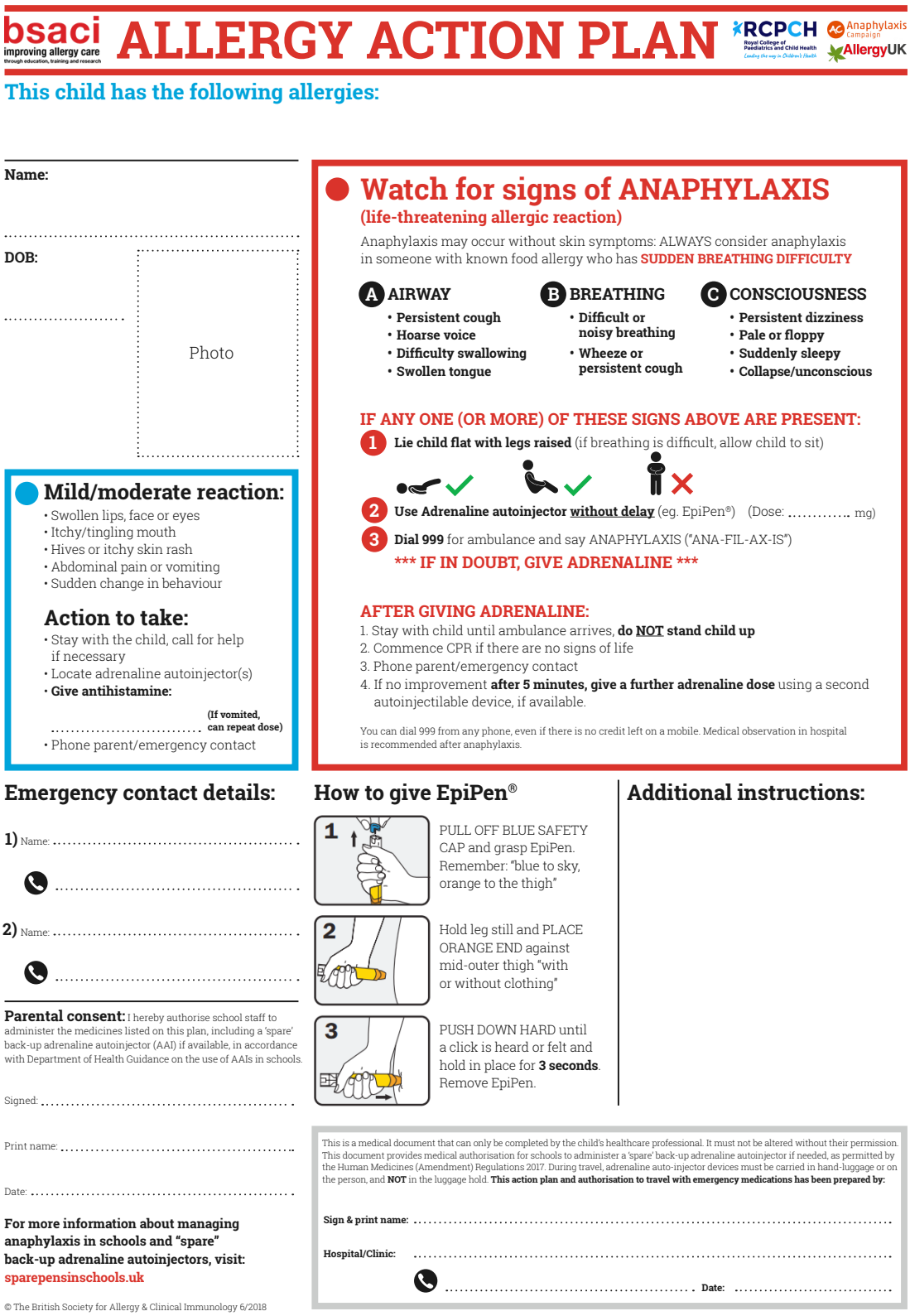
Table 5. Starting dose for OIT in Phase 1.

Prior to discharge home, each family will be provided with:

- A **symptom advice sheet** (including advice on avoiding intense physical exercise up to 2 hours after the dose intake).
- A **food allergy management plan** (example in Figure 4), including an emergency medicine kit containing non-sedating oral antihistamine, two adrenaline auto-injector devices and a salbutamol inhaler with spacer device. Families and participants will be instructed in the prompt recognition and treatment of allergic reactions, including adrenaline auto-injectors. The expiry date of auto-injection devices will be checked, and a prescription for replacements provided if needed.
- **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.
- **Dietary advice on CM avoidance**. Participants will be given instructions to avoid all CM or CM-containing foods during the study, with the exception of study immunotherapy doses. Up to 70% of children with CM allergy tolerate the allergen when extensively heated, such as in a cake or biscuit: the heating, in a wheat-containing matrix, significantly modifies CM allergenicity. Participants who are able to tolerate baked foods containing CM, will be requested to avoid all CM including in baked foods during the study, since uncontrolled exposure to CM protein in baked foods will impact upon treatment outcomes.
- A **home diary and instruction sheet**, which will specify to participants/parents what study dose to give their child, and on to which they will log all doses taken at home and any resulting adverse events. Subjects will be asked to record any symptoms, duration, timing in relation to immunotherapy dose and any exacerbating factors (e.g. exercise, excessive tiredness, viral illness). In the event of any symptoms, the family needs to contact the research team for advice.

At subsequent increases, the dose will first be given (by either the sublingual or oral route, depending on allocation) under medical supervision, followed by observation for at least one hour (2 hours if participant has previously experienced a delayed reaction). Prior to updosing, the supervising clinician will assess the participant to ensure:

- No significant allergic reactions to doses given in previous 2 weeks.
- No acute exacerbation of asthma in the past week.
- No concurrent systemic illness.



bsaci improving allergy care through education, training and research

ALLERGY ACTION PLAN

RCPCH Royal College of Paediatrics and Child Health · Anaphylaxis Campaign · AllergyUK

This child has the following allergies:

Name:

.....

DOB:

.....

Photo

Mild/moderate reaction:

- Swollen lips, face or eyes
- Itchy/tingling mouth
- Hives or itchy skin rash
- Abdominal pain or vomiting
- Sudden change in behaviour

Action to take:

- Stay with the child, call for help if necessary
- Locate adrenaline autoinjector(s)
- **Give antihistamine:**

..... **(If vomited, can repeat dose)**

- Phone parent/emergency contact

Watch for signs of ANAPHYLAXIS

(life-threatening allergic reaction)

Anaphylaxis may occur without skin symptoms: ALWAYS consider anaphylaxis in someone with known food allergy who has **SUDDEN BREATHING DIFFICULTY**

A AIRWAY	B BREATHING	C CONSCIOUSNESS
• Persistent cough	• Difficult or noisy breathing	• Persistent dizziness
• Hoarse voice	• Wheeze or persistent cough	• Pale or floppy
• Difficulty swallowing		• Suddenly sleepy
• Swollen tongue		• Collapse/unconscious

IF ANY ONE (OR MORE) OF THESE SIGNS ABOVE ARE PRESENT:

1. **Lie child flat with legs raised** (if breathing is difficult, allow child to sit)
2. **Use Adrenaline autoinjector without delay** (eg. EpiPen®) (Dose: ..... mg)
3. **Dial 999** for ambulance and say ANAPHYLAXIS ("ANA-FIL-AX-IS")

***** IF IN DOUBT, GIVE ADRENALINE *****

AFTER GIVING ADRENALINE:

1. Stay with child until ambulance arrives, **do NOT stand child up**
2. Commence CPR if there are no signs of life
3. Phone parent/emergency contact
4. If no improvement **after 5 minutes, give a further adrenaline dose** using a second autoinjectilable device, if available.

You can dial 999 from any phone, even if there is no credit left on a mobile. Medical observation in hospital is recommended after anaphylaxis.

Emergency contact details:

1) Name: .....

.....

2) Name: .....

.....

Parental consent: I hereby authorise school staff to administer the medicines listed on this plan, including a 'spare' back-up adrenaline autoinjector (AAI) if available, in accordance with Department of Health Guidance on the use of AAIs in schools.

Signed: .....

Print name: .....

Date: .....

For more information about managing anaphylaxis in schools and "spare" back-up adrenaline autoinjectors, visit:
sparepensinschools.uk

How to give EpiPen®

1. PULL OFF BLUE SAFETY CAP and grasp EpiPen. Remember: "blue to sky, orange to the thigh"
2. Hold leg still and PLACE ORANGE END against mid-outer thigh "with or without clothing"
3. PUSH DOWN HARD until a click is heard or felt and hold in place for **3 seconds**. Remove EpiPen.

Additional instructions:

This is a medical document that can only be completed by the child's healthcare professional. It must not be altered without their permission. This document provides medical authorisation for schools to administer a 'spare' back-up adrenaline autoinjector if needed, as permitted by the Human Medicines (Amendment) Regulations 2017. During travel, adrenaline auto-injector devices must be carried in hand-luggage or on the person, and **NOT** in the luggage hold. **This action plan and authorisation to travel with emergency medications has been prepared by:**

Sign & print name: .....

Hospital/Clinic: .....

..... **Date:** .....

Figure 4: Example of local national Allergy Management Plan

5.3.2.2 PREPARATION OF DOSES FOR PHASE 1 PRETREATMENT

The research team will prepare the doses for daily home administration during phase 1. On the initial escalation day visit, and at each subsequent updosing visit, the family will be provided with the required number of pre-labelled syringes, one for each day. Families will be asked to store the syringes in their home freezer at -18 to -22°C in a plastic container provided. Syringes will be taken out of the freezer 30 minutes prior to administration.

For children receiving ACTIVE treatment (groups S and O), the dose will contain the appropriate concentration of powdered milk (Protifar, Nutricia Ltd, Wiltshire, UK, containing 87.2 g cow's milk protein per 100g with equivalent casein/whey protein ratio as fresh milk). Children in group C will be given syringes containing a suspension of tapioca starch (Bob's Red Mill Natural Foods, Milwaukie, USA) as placebo, to maintain blinding (as needed). The same instructions will be followed for daily dose administrations for all participants.

Doses will be prepared in a designated food preparation area, following established food hygiene procedures. An unblinded member of the study research team (not be involved in the assessment of study outcomes) will prepare the doses in the first instance, according to a standard operating procedure. Each randomised participant will be allocated a labelled set of syringes, thus blinding of both the participant's families and local study team will be maintained.

5.3.3 HOME REACTIONS AND CONSEQUENTIAL CHANGES TO UPDOSING PROTOCOL

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, the following measures will be taken:

- Families will be instructed to follow their Allergy Management Plan (example in Figure 4), and to contact the study team.
- 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 provided and contact the study team on the Emergency number.

Once the study team has been informed, and following complete resolution of symptoms, the following plan of action will be recommended:

- For a single reaction consisting of wheeze, persistent cough, difficulty breathing, stridor or marked swallowing difficulties, the family must not give further doses without contacting the research team. The previous lower dose will be given the next possible day, preferably under medical observation on the Research Unit.
- For mild skin symptoms, itchy mouth, rhinoconjunctivitis or mild abdominal symptoms, reassurance to families will be provided and the current dose will be continued, with daily contact with the study team as needed.
- For persistent reactions for 3+days (within 2 hours of the immunotherapy dose) consisting of abdominal pain, rhinoconjunctivitis or skin symptoms, the local investigator should consider a dose reduction of 50% to be made until symptoms have mostly resolved.
- In the event of other symptoms that prove difficult to the child, the dose can be reduced at investigator's discretion.
- For recurrent symptoms, the local investigator may elect to offer participants an oral antihistamine during Phase 1, prior to dosing, to reduce allergic symptoms to immunotherapy doses, according to accepted clinical practice.

Where a dose reduction is required, the subsequent dose increase should be performed at least 2 weeks later at the Research Unit in hospital under supervision.

In the event of omitted doses:

- If 1-2 days of immunotherapy is missed, the subject can continue with the usual dose.
- If 3-4 days of immunotherapy are missed, the previous dose will be given until the subject can attend the research unit for repeat updosing.
- If more than 4 days are missed, dosing should stop until the subject can attend the research unit for repeat updosing.

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

5.4 INTERIM POST-PHASE 1 ASSESSMENT (AROUND 6 MONTHS)

Subjects will be invited to undergo a DBPCFC to CM following completion of Phase 1 to again determine their threshold of reactivity to CM, according to Table 2 (Section 5.2.2). The pre-challenge assessment and challenge protocol are described in Section 5.2. Both challenge visits should take place with 28 days of each other. Subjects will also undergo repeat skin prick testing and HRQL assessments as described in section 5.8. Participants will continue their Phase 1 daily dosing until phase 2 is commenced.

5.5 PHASE 2 - CONVENTIONAL ORAL IMMUNOTHERAPY

In phase 2, subjects will receive daily oral CM doses at home in an open, unblinded manner. Dose increases will be performed under medical supervision on the research Unit following the regime depicted in Table 6 ("conventional OIT"). Phase 2 will last 6 months (26 weeks) from the first updosing visit of Phase 2.

The protein content of supermarket-sourced commercial milk is 3.4g/100ml in UK and 3.0g/100ml in Spain. Therefore, the volume of milk to be delivered will vary slightly, by study site, in order to achieve the same dose of CM Protein across study sites (Table 6).

PHASE 2 UPDOSING PROTOCOL (CONVENTIONAL OIT)						
Step	**Target CM protein (mg)**	**Supermarket milk (ml)**		**Milk preparation**	**Final dose (ml)**	
		UK	**SPAIN**		**UK**	**SPAIN**
1	0.04	0.0012	0.0013	Diluted 1:100	0.125	0.15
2	0.08	0.0024	0.0027		0.25	0.3
3	0.2	0.0059	0.0067		0.6	0.7
4	0.4	0.0118	0.0133	Diluted 1:10	0.12	0.15
5	1	0.0294	0.0333		0.3	0.35
6	2.5	0.0735	0.0833		0.75	0.8
7	6	0.175	0.2	Undiluted milk	0.2	0.2
8	15	0.45	0.5		0.4	0.5
9	30	0.9	1.0		0.9	1.0
10	60	1.75	2.0		1.75	2.0
11	135	4.0	4.5		4.0	4.5
12	255	7.5	8.5		7.5	8.5
13	510	15	17		15	17
14	1020	30	34		30	34
15	2040	60	68		60	68
16	4080	120	136		120	136

Table 6. Phase 2 updosing protocol (conventional OIT)

Although there are 16 steps to the OIT protocol in Phase 2, we expect at least 95% of participants to be able to commence the protocol at Step 7+ (i.e. with undiluted supermarket-sourced milk), and thus complete the full protocol within the 6 months allocated:

- Over two thirds of participants (i.e. those in groups S and O) are expected to reach 60mg CM protein by the end of phase 1, which corresponds to step 10.
- On the basis of population threshold data for cow's milk, we expect 90% of participants (even those receiving placebo in phase 1) to tolerate step 7 (6mg CM protein).

The inclusion of Steps 1-6 are to allow those few participants who remain very sensitive to low doses of CM protein to continue to participate in the study.

5.5.1 INITIAL UPDOSING / DOSE ESCALATION DAY

The starting dose of OIT in phase 2 will be individualised based on the outcome of the interim DBPCFC at 4-7 months, as shown in Table 7. The NOAEL (No Observed Adverse Event Level or highest dose not triggering symptoms) will be used as the tolerated dose. Mild transient symptoms (e.g. itchy mouth) to the reference dose (transient COFAR Grade 1 symptoms (Table 9) might be acceptable.

Tolerated dose (NOAEL) at 6 months DBPCFC	**Initial dose for Phase 2 OIT**		**Max dose at Initial Escalation day**	
	Step	**CM Protein (mg)**	**Step**	**CM Protein (mg)**
<0.5mg (i.e. 1st dose reactor at FC)	1	0.04	6	2.5
0.5mg	3	0.2	6	2.5
3mg	4	0.4	7	6
10mg	6	2.5	8	15
30mg	9	30	n/a	n/a
100mg	10	60	n/a	n/a
300mg	10	60	n/a	n/a
1000mg or more	11	135	n/a	n/a

Table 7. Starting dose for OIT in Phase 2.

Participants starting phase 2 OIT at steps 1-6 will be offered to attend an escalation day visit at which up to 6 consecutive doses will be given by mouth at 20-30 minutes intervals under medical supervision on the Research Unit. As described in **5.3.2.1** (phase 1 escalation day), the supervising clinician will assess the participant to ensure suitability prior to dosing. If a given dose triggers symptoms that cause discomfort to the participant or 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 on a daily basis for a minimum of 10 days, until the next increase is performed.

At the discretion of the local investigator, participants commencing phase 2 OIT at step 9 or above do not need to attend for a separate escalation day:

- If the 2nd DBPCFC visit (at 6 months) is ACTIVE, then they can start the OIT dose the next day at home, unless there is doubt as to the dose tolerated at challenge (in which case a separate initiation visit should take place).
- If the 2nd DBPCFC visit is PLACEBO, then the initial OIT dose(s) can be given at the end of the 2 hour observation period of the challenge, where time permits.

Participants will then be asked to have their daily dose at home for a minimum of 10 days until the next increase is performed.

Prior to discharge home, patients will receive instructions including symptoms management and reporting as described in **5.3.2.1**.

5.5.2 UPDOSING (PHASE 2)

Updosing in Phase 2 will be performed in a similar manner to that in Phase 1 (section **5.3.2.1).** At each updosing visit, the dose will first be given under medical supervision, followed by observation for at least one hour (2 hours if participant has previously experienced a delayed reaction). Participants will then take that dose daily at home for a minimum of 10 days, until the next updosing visit. Once the target dose is reached (step 16), participants will continue this dose on a daily basis until the final assessment at 1 year has been completed.

5.5.3 DOSE PREPARATION FOR PHASE 2 OIT

Locally-sourced supermarket milk will be used for doses. Parents will receive written instructions on how to give doses at home.

The majority of participants are expected to start home dosing beyond step 9 i.e. with undiluted pasteurised milk available at supermarkets. For those few participants where diluted doses (steps 1 to 6) are needed, these will be provided with pre-labelled individual syringes containing the daily dose for the appropriate step. Families will be asked to store the syringes in their home freezer at -18°C in a plastic container. Each syringe will be used for a single dose, with the syringe taken out of the freezer 30 minutes prior to administration.

5.5.4 CHANGES TO THE PROTOCOL DUE TO REACTIONS

The same criteria and measures described in 5.3.2.4 for phase 1 will be followed in phase 2.

5.5.5 CONCOMITANT MEDICATION DURING PHASE 1 & 2

Participants may continue usual medications, including those taken for asthma, rhinitis, and eczema during the study. Antihistamines must be stopped prior to screening and DBPCFC visits as per section 5.2.1. Participants (or their parents) will be asked to document any use of medication (including rescue medication) in home diary cards.

5.5.5.1 MANAGEMENT OF RECURRENT SYMPTOMS

While treatments for recurrent AEs are permitted (e.g. H1-antihistamines), these medicines should not be routinely used in advance of symptoms due to OIT. Antihistamines may be taken after review by the local chief investigator for short-term symptomatic relief only, unless required for the treatment of allergic rhinitis during the study. If started, the use of these medications should be minimized, and then discontinued at the earliest opportunity.

Symptomatic treatment not involving antihistamine for chronic/recurrent AEs is permitted (subject to compliance with the exclusion criteria for the study), but should be used only where dose reduction has not on its own been effective. Any medicine instituted for treatment of symptoms (AEs) related to OIT doses must be withdrawn by 4 weeks prior to the exit DBPCFC at 12 months.

5.6 12 MONTH ASSESSMENT / EXIT DBPCFC

All subjects will be offered a DBPCFC to CM and receive further dietary advice.

5.6.1 DBPCFC

All subjects will be invited to undergo a further DBPCFC to CM at approximately 12 months as described in 5.2. Both DBPCFC visits should take place between 24-30 weeks after commencing Phase 2 OIT. No OIT dose should be administered on the same day as a challenge visit. The purpose of this challenge is to determine the effect of the treatment in participants' threshold of reactivity. Successful desensitization will be defined as a negative outcome to a cumulative dose of 8g CM protein.

5.6.2 DIETARY ADVICE

Future advice will be provided following the second visit at the 12 months DBPCFC regarding CM consumption, based on the threshold of reactivity at DBPCFC as follows:

- Participants reacting at doses below 1.4g CM protein cumulative will be assessed by the study investigators and provided with advice relating to CM exposure according to local clinical protocol. Many of these participants may be able to tolerate small amounts of CM in baked foods: where appropriate, this will be assessed as part of the participant's routine clinical care.
- Participants able to tolerate at least 1.4g CM protein will be asked to continue their daily CM exposure at the same dose taken immediately prior to the 12 month DBPCFC. This regular CM intake will ensure that the effect of the treatment is maintained. Participants (and their families) will also be given appropriate advice allowing them to include CM-containing products up to the amount they tolerate at the 12 month DBPCFC.

All subjects will also undergo repeat skin prick testing and HRQL assessments at 12 months.

5.7 HRQL ASSESSMENT AT 15 MONTHS

HRQL will also be assessed 3 months after the exit DBPCFC. This can be done by email/online if preferred by the participant and their family.

5.8 OTHER ASSESSMENTS

5.8.1 ALLERGY SKIN PRICK TESTING

Skin prick testing (SPT) will be performed using ALK lancets (ALK-Abelló, Hørsholm, Denmark) on the volar side of the forearm of study participants, according to local standard operating procedures consistent with national guidelines.[14]

SPT will be measured at 15 minutes: the wheal will be traced around using pen and then an imprint taken with cellophane tape (e.g. sellotape); mean wheal diameter (average of longest length and its perpendicular width) will be measured.

SPT will be performed at the initial screening visit, 6 and 12 months after starting immunotherapy, using:

- Commercial extracts of whole CM and casein (ALK-Abelló, Hørsholm, Denmark), together with histamine positive and saline negative controls.
- Titrated SPT (end-point titration, EPT), to fresh* CM at the following concentrations:
    - neat, and
    - diluted (with sterile saline) at 1/10, 1/100, 1/1000, 1/10,000, 1/100,000

In addition, at *each* initial DBPCFC visit, SPT will be performed to fresh* CM and commercial CM extract (together with positive and negative control) prior to challenge. The wheal response will then be measured at approximately 30 minute intervals thereafter, until the wheal has reduced to 3mm or less, or the patient is discharged.

The time taken for the mean wheal size (elicited by SPT using neat milk or commercial extract) to reduce to 3mm (PT3) will be calculated using the following formula:

$$PT3 = T_1 + (3-W_1) \times \frac{T_2 - T_1}{W_2 - W_1}$$

where: T_1 = time when wheal diameter has reduced to < 3mm
W_1 = size of wheal at T_1.
T_2 = time when wheal diameter has reduced to just above 3mm.
W_2 = size of wheal at T_2.

*Fresh CM is not readily available in Spain. Therefore, SPT will be performed to freshly thawed cow's milk sourced from the UK market. Aliquots of fresh CM from the same batch of CM will be prepared in both centres and the frozen for use throughout the study for SPT. This has the added advantage of the same CM product being used throughout in both centres. We have previously demonstrated that a single freeze-thaw cycle of fresh CM does not affect SPT responses to CM.

[14] http://www.bsaci.org/Guidelines/Skin_Prick_Testing.pdf

5.8.2 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 abbreviated versions of disease-specific validated "food-allergy quality of life questionnaire" (FAQL-Q) in its parental,[15] child[16] and teenage[17] forms at five time points during the study:

i. baseline, at screening
ii. baseline, after completion of the baseline DBPCFC
iii. prior to the DBPCFC in between Phase 1 and Phase 2, at around 6 months
iv. prior to the exit DBPCFC after Phase 2.
v. 3 months after the exit DBPCFC (15 months visit).

These new abbreviated versions have been validated (data submitted for publication). We will also assess the impact of the child's food allergy on parent QoL using the FAQL-Parent Burden form (which has been validated in a UK population).[18]

The FAQL-questionnaires are based on a 7-point Likert scale and changes greater than 0.5 points over time have been defined as clinically significant.[19] Previous studies have reported a positive effect on QoL following OIT to CM from the parents' perspective.[20] However, there are no controlled studies which have addressed the impact on QoL as perceived by the participants themselves. This is a significant knowledge gap, since there is a concern that while parents report an improved HRQL when their child undergoes immunotherapy the child themselves may experience a worsening of HRQL measures due to frequent side effects and need to alter their activities following immunotherapy doses.

In line with current expert opinion, and 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 versions of the QoL assessments unless there are difficulties with comprehension which preclude this.

We will also assess HRQL using EQ-5D,[21] a standardized instrument developed by the EuroQol Group as a measure of health-related quality of life that can be used in a wide range of health conditions and treatments, and is used by organisations such as the UK National Institute for Health and Care Excellence (NICE) to determine Quality-adjusted life year (QALYS). The EQ-5D consists of a descriptive system and the EQ Visual Analogue Scale, VAS. The descriptive system comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ VAS records the patient's self-rated health on a vertical visual analogue scale. This can be used as a quantitative measure of health outcome that reflects the patient's own judgement. The scores on these five dimensions can be presented as a health profile or can be converted to a single summary index number (utility) reflecting preferability compared to other health profiles. EQ5D will be assessed at the same time points as FAQLQ.

---

[15] DunnGalvin A, Cullinane C, Daly DA, Flokstra-de Blok BM, Dubois AE, Hourihane JO. Longitudinal validity and responsiveness of the Food Allergy Quality of Life Questionnaire - Parent Form in children 0-12 years following positive and negative food challenges. Clin Exp Allergy. 2010;40:476-85.

[16] Flokstra-de Blok BM, DunnGalvin A, Vlieg-Boerstra BJ, et al. Development and validation of a self-administered Food Allergy Quality of Life Questionnaire for children. Clin Exp Allergy. 2009;39:127-37.

[17] Flokstra-de Blok B, DunnGalvin A, Vlieg-Boerstra BJ, et al. Development and validation of the self-administered Food Allergy Quality of Life Questionnaire for adolescents. J Allergy Clin Immunol. 2008;122:139-44, 144.e1-2.

[18] Knibb RC, Stalker C. Validation of the Food Allergy Quality of Life-Parental Burden Questionnaire in the UK. Qual Life Res. 2013;22:1841-9.

[19] Jaeschke R, Singer J, Guyatt GH. Measurement of health status. Ascertaining the minimal clinically important difference. Control Clin Trials 1989; 10:407–15.

[20] Carraro S, Frigo AC, Perin M, et al. Impact of oral immunotherapy on quality of life in children with cow milk allergy: a pilot study. Int J Immunopathol Pharmacol. 2012;25:793-8.

[21] https://euroqol.org/eq-5d-instruments/

Finally, we will include a brief self-efficacy assessment which evaluates participants (and their parents') ability to treat and manage their allergy, and how this changes during the trial. This questionnaire has been validated and used in a previous immunotherapy study in peanut allergy.

In subjects who are withdrawn from the study due to a significant allergic reaction, we will contact the family at 3 months later to repeat HRQL assessments. This will allow us to assess the impact of 'failing' immunotherapy on the young person and their family.

5.8.3 LABORATORY ASSESSMENTS

The development of tolerance in immunotherapy (whether to food or aeroallergens) is associated with several immunological changes, including decreases in CM-specific IgE levels with concomitant increases in IgG4 levels, reduced basophil and Th-2 cytokine responses to peanut stimulation and upregulation of IL-10-producing T-regulatory cells.[22]Also specific IgA in saliva has been reported as a potential mechanism involved in effective SLIT to foods, at least to peanut.[23]

It is therefore important for this study to include mechanistic assessments to better understand the ability of the different immunotherapy strategies tested to induce immune changes leading to desensitization. Additionally, various characteristics such as baseline allergen-specific IgE might be useful to predict the outcome of immunotherapy to foods. For instance, IgE binding to particular sets of CM peptides was found to be useful to predict the safety and efficacy of CM-OIT in a preliminary study.[24] The mechanistic work in this study will allow for markers of safe and successful desensitisation to be assessed, in order to improve the efficacy and safety of future immunotherapy protocols.

We will include a series of laboratory assessments in all subjects, allowing longitudinal comparisons amongst the different study groups as well as the evaluation of biomarkers that may identify successful desensitization to CM. These assessments will include:

- Changes in serum specific antibodies pre- and post-immunotherapy: Total IgE; specific IgE and IgG4 to CM and CM components (casein, alphalactalbumin and betalactoglobulin).
- CM-specific IgA in saliva using ELISA
- Identification of serum factors associated with the development of desensitization during CM immunotherapy.
- IgE and IgG_4 binding to CM epitopes, as assessed using a novel peptide microarray platform.
- Changes in T and B cell populations and their immune profile during CM immunotherapy including allergen-specific proliferation of T-cell subtypes (including T helper and regulatory T cells) and changes in immunoglobulin repertoire by CM-specific B-cells (London site only).

These assessments will be performed on blood samples taken at baseline, 6 and 12 months. Saliva samples will be collected using saliva oral swabs (Salivette, Sarstedt). Laboratory work will be initially performed at the National Heart & Lung Institute, Imperial College London, although some assays may be performed elsewhere in the UK and EU where lack of local expertise necessitates this. Consent will be requested for specimens to be stored for future use in new assays to measure immune responses or biomarkers.

[22]Gorelik M, Narisety SD, Guerrerio AL, et al. Suppression of the immunologic response to peanut during immunotherapy is often transient.J Allergy ClinImmunol. 2014; doi: 10.1016/j.jaci.2014.11.010.
[23] Kulis M, Saba K, Kim EH, et al. Increased peanut-specific IgA levels in saliva correlate with food challenge outcomes after peanut sublingual immunotherapy. J Allergy Clin Immunol. 2012 Apr;129(4):1159-62.
[24] Martínez-Botas J, Rodríguez-Álvarez M, Cerecedo I, et al. Identification of novel peptide biomarkers to predict safety and efficacy of cow's milk oral immunotherapy by peptide microarray. Clin Exp Allergy. 2015 Jun;45(6):1071-84.

5.8.3 ORAL AND FAECAL MICROBIOME ASSESSMENTS

As an exploratory analysis, we will collect both saliva and stool samples from participants at one of the baseline visits in order to assess oral and faecal microbiome in this unique population of children with persistent milk allergy. In conjunction with Nutricia Research / University of Utrecht (Netherlands), the following samples will be collected at the following time points from both participants but also, where consent is forthcoming, household siblings within 4 years of age older/younger than the participant with CM allergy taking part in the study.

	Oral microbiome	**Faecal microbiome**
Assessed at the following time points:		
• Index case (study participant)	Baseline After Phase 1 (pre DBPCFC) After Phase 2 (pre DBPCFC)	Baseline only
• Controls (household siblings)	Baseline only	Baseline only
Method of collection	In study participants, the swab for salivary IgA will be collected first (see 5.8.3) and then the mouth rinsed with water. Saliva for microbiome analyses will be collected using Oracol saliva collection system (Malvern Medical) as follows: • Rinse mouth with drinking water 10 minutes pre sampling • Place the sponge in the mouth between teeth and cheek for 2 minutes. Avoid changing position of the sponge, but if the sponge adheres to the cheek, rotate the sponge a quarter turn. If desirable, the sponge can be transferred to the other side of the mouth. After collection, store at -80°C until analysis. For siblings, the collection swab will be given to parents to collect saliva at home immediately prior to a study appointment.	Families will be given a sample collection pot(s) at screening and specimen bag and asked to bring the sample with them on one of the DBPCFC visits.

Table 8. Collection of samples for microbiome assessment

The use of household siblings as a control in the assessment of the oral and faecal microbiome is needed in order to control for environmental exposures which can significantly modify the microbiome and impair the assessment of changes in microbiota which might be due to atopic/allergic disease and how this might change during the course of oral immunotherapy.

Parents will be asked to collect these non-invasive samples from any household siblings under 16 years of age (within 4 years of age of the study participant with cow's milk allergy) so long as consent is provided by the parent/legal guardian and verbal assent by the sibling. Parents will be instructed not to force siblings to provide saliva/stool samples where verbal assent is not forthcoming.

Parents will also be asked to provide a minimum dataset of information relating to sibling controls:

- Age and gender
- Atopic status: eczema / asthma / food allergies / allergic rhinitis
- Brief dietary history of exposure of exposure to dairy products
- Any medical or surgical conditions affected the gastrointestinal system.

These data will be collected on a CRF and only identifiable through linkage to the index study participant.

5.9 STUDY COMPLETION

The study duration is approximately 15 months following enrolment. The study will be considered complete following enrolment of the last patient and completion of the study procedures in that patient.

At the end of the study, participants will be re-integrated into the routine clinical allergy service for ongoing care.

5.9.1 EARLY STUDY TERMINATION

An Independent Data Monitoring Committee (IDMC) will assess study safety on a regular basis. The IDMC has the authority to suspend the study, or request withdrawal of a particular participant at any stage if it concludes there are significant safety concerns affecting a single participant or the study as a whole.

Recruitment to the study and further CM challenges and updosing will be suspended pending review by the IDMC in the event of:

- any death
- a participant being admitted to the intensive care unit for a study-related adverse event
- a participant experiencing a life-threatening anaphylactic reaction to an immunotherapy dose.

The IDMC will consider the circumstances and make a recommendation as to whether to terminate the study or continue with or without a protocol amendment.

6. ADVERSE EVENT REPORTING

6.1 DEFINITIONS

An **adverse event** (AE) is any occurrence or worsening of an undesirable or unintended sign symptom. laboratory finding or disease that occurs during participation including occurrences that are not necessarily caused by or related to a study intervention. 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** (SAE) is defined as any adverse event that suggests a significant hazard. This includes but is not limited to any of the following:

1. Death: Any death that occurs during the study must be reported whether considered treatment related or not.

2. A life-threatening event: 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. In line with guidance from the Food and Drug Administration (FDA) for Industry and Investigators[25], "it does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death."

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. Important AEs that are not immediately life-threatening or do not result in death or hospitalization but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

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"**.

[25] 2012 Food and Drug Administration (FDA) Guidance for Industry and Investigators, "Safety Reporting Requirements for INDs and bioavailability studies

All episodes of anaphylaxis occurring in response to a dose of SLIT/OIT will be treated as an *AE "of interest"* and reviewed by the Trial Management team.

The following criteria will constitute an SAE:

- Anaphylaxis occurring at in-hospital challenge *requiring* three or more doses of intramuscular adrenaline
- Anaphylaxis occurring in response to a dose of SLIT/OIT outside the hospital environment, requiring more than one dose of intramuscular adrenaline. **Such reactions will be classified as a SERIOUS ADVERSE REACTION**.
- Clinician-confirmed diagnosis of eosinophilic oesophagitis (with positive histology).

6.2 DOCUMENTATION OF ADVERSE EVENTS

All adverse events will be recorded on a specifically designated case report form (CRF) 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.

All serious adverse events (SAEs) will be reported on a SAE report form in addition to CRFs. Safety data will be reviewed at least every four months by the Independent Data Monitoring Committee (IDMC). The IDMC has the authority to recommend withdrawal of a study participant or termination of the trial because of safety findings.

All SAEs should be reported to the Research Ethics Committee where, in the opinion of the Chief Investigator, the event was:

- 'related' i.e. resulted from the administration of any of the research procedures; and
- 'unexpected' i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the event using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs. Any episode of anaphylaxis occurring outside DBPCFC challenge visits during the study, including those not classified as SAE, will be reported to the IDMC.

6.3 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

Adverse events will be graded by the study site according to the criteria set forth in the NCI-CTCAE Version 3.0.[26] This document provides a common language to describe levels of severity to analyse and interpret data and to articulate the clinical significance of all adverse events:

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 recorded and graded whether they are or are not related to disease progression or treatment. The NCI-CTCAE grades will be the primary source for scoring.

[26]http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf

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 9. These can be used to inform as to the appropriate NTI-CTCAE grade.

<table>
<tr><th>Grade 1 –

Transient</th><th>Grade 1-

Mild</th><th>Grade 2 –

Moderate</th><th>Grade 3 –

Severe</th><th>Grade 4 –

Life Threatening</th><th>Grade 5 -

Death</th></tr>
<tr><td>Mild symptoms, <20mins.

No impact on activity, no intervention needed.</td><td>No or mild discomfort (< 48 hours).

No or minimal medical intervention/ therapy required.</td><td>Symptoms produce mild to moderate limitation in activity.

Some assistance may be needed; no or minimal intervention/ therapy is required.</td><td>Marked limitation in activity.

Some assistance usually required; hospitalisation is possible.</td><td>Extreme limitation in activity.

Significant medical intervention is required; hospitalisation is probable.</td><td>Death</td></tr>
<tr><td colspan="2">These symptoms may include pruritus, periorbital or facial angioedema, swelling or rash, mild abdominal discomfort, or other transient symptoms.</td><td>Hospitalization is possible but unlikely. These symptoms may include persistent hives, wheezing without dyspnea, abdominal discomfort / increased vomiting, or other symptoms</td><td>Symptoms may include broncho-spasm with dyspnoea, severe abdominal pain, throat tightness with hoarseness, transient hypo-tension among others. Parenteral medication(s) are usually indicated.</td><td>Symptoms may include persistent hypotension and/or hypoxia with resultant decreased level of conscious-ness associated with collapse and/ or incontinence or other life-threatening symptoms.</td><td></td></tr>
</table>

Table 9: CoFAR Specific Grading System for Allergic Reactions

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 assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in Table 10. If any doubt about the causality exists the investigator will discuss with the Chief Investigator. In the case of discrepant views on causality between the investigator and others, all parties will discuss the case.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition. other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition. other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

Table 10: Assignment of causality for adverse event

7. STATISTICS AND DATA ANALYSIS

7.1 SAMPLE SIZE ESTIMATION

The primary objective of the study is to assess the impact of a 6 month SLIT pretreatment phase on the safety of conventional OIT in children with persistent cow's milk allergy. The primary outcome is the proportion of participants experiencing adverse events classified as mild non-transient symptoms or more severe (see table 9) during CM-OIT (phase 2), in those who have received SLIT pretreatment (group S) compared to placebo (group C). As a secondary outcome, we will assess whether any effect of SLIT is dependent on the sublingual route, by including a third treatment group who have the same dosing regimen as the SLIT group but take the dose orally, omitting the sublingual phase (group O).

The expected natural resolution rate in CM allergic children beyond preschool age is less than 10% in a 6 months period.[27] Up to 70% of children undergoing conventional OIT for CM allergy experience mild, transient symptoms e.g. oropharyngeal itch [20.23.38]. A previous study in 9 peanut-allergic subjects showed a 75% relative reduction in mild non-transient symptoms (or symptoms of greater severity) during OIT as a result of the SLIT pretreatment.[28] Assuming 10% loss-to-follow-up, we calculate that a sample size of 66 participants (allocated 1:1:1 to the treatment groups) will detect a difference in the rate of children experiencing mild, non-transient symptoms from 70% in the OIT-alone group to 18% in the group undergoing SLIT pre-treatment prior to OIT (i.e. a 75% reduction), with 80% power at a significance level of $p<0.01666$ (Bonferroni correction) to allow for three pairwise comparisons (to ensure the overall risk of type I error is under 5%).

7.2 STATISTICAL ANALYSIS PLAN

Data will be collected on electronic clinical records or paper CRFs which include the patients name, DOB and hospital number. Participants will be assigned a study number which will be noted on all records. Data will then be entered on to a password-protected computer database on the secure hospital IT system; only the study number will be used to identify the patient data.

De-identified data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study including the follow-up period.

The primary analysis will be performed on an intention-to-treat basis, 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.

7.2.1 ANALYSIS OF PRIMARY ENDPOINT

The primary analysis will compare the proportion of participants experiencing adverse events classified as mild non-transient symptoms or more severe (see table 9) during CM-OIT (phase 2), in those who have received SLIT pretreatment (group S) compared to placebo (group C) using a two-sided Fisher's exact test at a $p<0.05$ level of significance.

[27] Wood. J Allergy Clin Immunol. 2013 March ; 131(3): 805–812
[28] Narisety et al. J Allergy Clin Immunol. 2015 May;135(5):1275-82.e1-6.

7.2.2 ANALYSIS OF SECONDARY ENDPOINTS

The following secondary analyses will be performed using two-sided testing for non-parametric data at a $p<0.05$ level of significance:

- The incidence of adverse events experienced (including rate of withdrawals, and anaphylaxis/ adrenaline use during updosing) between groups S, O and C in phases 1 and 2. Statistical differences will be determined using Fisher's exact test for proportions or using non-parametric tests for graded reactions. Logistic regression will be used to assess the influence of age, gender, asthma status and prior history of anaphylaxis amongst other factors on the incidence of adverse events.
- Efficacy defined at DBPCFC as the proportion of study participants experiencing:
  - No symptoms (or only mild transient symptoms as per table 9) to 8 grams CM protein (approx. 250mls fresh milk) ("Complete desensitisation")
  - No symptoms (or only mild transient symptoms as per table 9) to at least 1.4 grams CM protein (approx. 45mls fresh milk) ("Partial desensitisation")
  - At least a 10-fold increase in eliciting dose (defined as the lowest dose which elicits objective symptoms or signs at challenge).

    …at 6 and 12 months in the different treatment groups, using a two-sided Fisher's exact test at a $p<0.05$ level of significance.
- This will include a comparison of the efficacy of SLIT compared to the same dose administered orally without a sublingual phase (to address hypothesis 2), and its effect on the subsequent safety of OIT.
  - Immunological outcomes (titrated skin prick test, serum IgE) at baseline, 6 and 12 months.
  - Change in health-related quality of life (HRQL) measures at 6, 12 and 15 months, as assessed by validated questionnaires (FAQLQ, FAIM, EQ-5D) in study participants and their parents.
- Change in health-related quality of life (HRQL) measures at 6, 12 and 15 months, as assessed by validated questionnaires (FAQLQ, FAIM, EQ-5D) in study participants and their parents.
- Immunological outcomes (titrated skin prick test, serum IgE) at baseline, 6 and 12 months.
- The trend in CM-peptide binding over time as well as the potential influence of baseline peptide binding on the outcome of CM-IT will be assessed using a bioinformatics approach as in previous studies.[29]

Secondary analyses will include a comparison of the safety/efficacy of SLIT compared to the same dose administered orally without a sublingual phase (to address hypothesis 2), and its effect on the subsequent safety/efficacy of OIT.

7.2.3 INTERIM ANALYSIS

There will be no formal interim analysis, however the IDMC will review safety data at least every 6 months.

[29] Martínez-Botas J, et al. Clin Exp Allergy. 2015 Jun;45(6):1071-84.

8. ADMINISTRATIVE AND REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Chief Investigator will obtain the required approvals from the relevant Research Ethics Committee. The study will be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly. Helsinki 1964 and later revisions.

8.2 INFORMED CONSENT AND PARTICIPANT ASSENT

Consent to enter the study must be sought for each participant only after a full explanation has been given an information leaflet offered and time allowed for consideration. Signed consent from the parent/legal guardian should be obtained. Participant assent will also be sought. The right of the parent/guardian to refuse to participate without giving reasons must be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3 MHRA EXEMPTION

Following discussions with MHRA it has been confirmed that this study is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC thus no submission to the Clinical Trials Unit at the MHRA is required. The cow's milk products used are food products and are not presented as a medicine (e.g. in a pharmaceutical form). The study is therefore exempt from MHRA requirements for a clinical trial. This confirmation is reproduced in Appendix 2.

8.4 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study under the Data Protection Act.

8.5 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.6 SPONSOR

Imperial College London will act as the main Sponsor for this study.

8.7 FUNDING

Funding has been secured from the J P Moulton Charitable Foundation for the London clinical site. The Madrid site is supported by grants from Sociedad Española de Alergología e Inmunología Clínica (SEAIC) and Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica (SEICAP), which is also supporting some of the immunological assays.

8.8 PROTOCOL COMPLIANCE

Every effort will be made to adhere to the study protocol and participants who are non-compliant with the protocol will be withdrawn after review and agreement between the 2 principle investigators. Protocol deviations may be captured from a variety of different sources including CRFs, EPR, communications and updates. The sponsor will maintain a log of the non-compliances to ascertain if there are any trends which need to be escalated. The sponsor will assess the non-compliances and action a timeframe in which they need to be dealt with. Each action will be given a different timeframe dependent on the severity. If the actions are not dealt with accordingly, the Research Compliance Office will agree an appropriate action, including an on-site audit.

8.9 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

9 STUDY MANAGEMENT

Trial Steering Committee

A Trial Steering Committee (TSC) will be established with an independent Chair plus at least one independent specialist doctor in allergy, and a parent of a food-allergic child. The TSC will convene prior to study initiation, and approximately every 4 months during the study. The major roles of the TSC will be:

- To make recommendations to ensure successful and safe delivery of the study
- To evaluate progress against the agreed timetable and deliverables
- To develop and implement successful communication between the study staff and external stakeholders.
- To determine frequency of IDMC meetings

A copy of all TSC minutes will be supplied to the Sponsor.

Independent Data Monitoring Committee

The primary responsibility of the IDMC will be safety of study participants. The role of its members is therefore to monitor safety data and make recommendations to the Chief Investigator and Sponsor on whether there are any ethical or safety reasons as to why the trial should not continue. The IDMC will comprise: an independent chair and two independent specialist clinicians. All SAE, SAR and SUSARs will be promptly reported to the IDMC by the study team. Both the TSC and IDMC will be provided with regular updates on outcomes of DBPCFC and AEs due to OIT doses. The IDMC will convene every 4-6 months, or more frequently if requested by the study team.

The day-to-day management of the study will be co-ordinated through Dr Bettina Duca at Imperial College London.

10 PUBLICATION POLICY

Results of this study will be published in scientific peer-reviewed literature relevant to allergic disease. Members of the Trial Steering Committee and the IDMC will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy.

APPENDIX 1: MANAGEMENT OF ANAPHYLAXIS

Local protocols will be followed for any participant requiring treatment for anaphylaxis.

In the event of refractory anaphylaxis (requiring over 2 doses of IM adrenaline or at investigator initiation), consideration will be given to initiate an adrenaline infusion according to a published protocol (reproduced below). This will be initiated ONLY under the direct supervision of a consultant experienced in the management of anaphylaxis.

The adrenaline infusion should be delivered by a syringe pump and not via a volumetric pump. The infusion can be administered through a dedicated peripheral line (to avoid inadvertent bolus dosing with other drugs/fluids) but the cannula should be sited in a large vein.

ADRENALINE INFUSION GUIDELINE FOR ANAPHYLAXIS

1 PREPARATION

- Requires continuous physiological monitoring **(ECG, SpO2, BP 3-5 minutely)**
- Give via an **infusion pump** through a **dedicated line**, or piggybacked with **anti-reflux valves on all other lines** to prevent the adrenaline going back up into another fluid bag instead of into the patient
- BEWARE infusions on the same side as a BP cuff; frequent BP measurements may interfere with the infusion
- FIRST BAG: **1mg adrenaline in 100 mL saline = 0.01mg/mL (1:100,000)**
  i.e. 1 ml/kg/Hr gives the equivalent of a 0.01 mg/kg dose over 1 hour (0.17 ug/kg/min)

2 INITIATION & ADJUSTMENT

- **Start at 0.5-1 mL/kg/Hr** (30-100 mL/Hr in adults) depending on reaction severity:
  Moderate severity: 0.5 mL/kg/Hr Severe (hypotensive or hypoxic): 1 mL/kg/Hr
- **Titrate** up or down according to response, aiming for the lowest effective infusion rate
  Allow for a short elimination half-life; steady state is reached 5-10 minutes after a change in the infusion rate
- **Tachycardia, tremor, and pallor with a normal or raised blood pressure are signs of adrenaline toxicity:**
  Reduce the infusion rate (if toxicity is severe, stop the infusion briefly before recommencing at a lower rate)
- **The safe maximum rate of adrenaline infusion** is unknown, but is probably <1 ug/kg/min (6mL/kg/Hr of the above solution of 1mg in 100 mL).

3 DE-ESCALATION AND CESSATION

- **As the reaction resolves, an infusion that was previously therapeutic can start to have toxic effects:** Therefore, when features resolve begin reducing the infusion, aiming for around half the starting rate if possible
- 60 minutes after the resolution of all symptoms and signs, wean the infusion over another 30 minutes and stop; watch closely for reaction recurrence

APPENDIX 2 MHRA EXEMPTION

RE: SCOPE - Food allergy study

Clinical Trial Helpline <ctdhelpline@mhra.gsi.gov.uk>

Wed 22/02/2017 14:24

To:Turner, Paul J <p.turner@imperial.ac.uk>;

Notification that a Clinical Trial Authorisation (CTA) is not required

Dear Dr Turner

Thank you for your email dated 17 February 2017.
I can confirm that your proposal is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC and no submission to the Clinical Trials Unit at the MHRA is required.

Kind regards

Clinical Trial Helpline

MHRA

Medicines & Healthcare products Regulatory Agency

From: Turner, Paul J [mailto:p.turner@imperial.ac.uk]
Sent: 17 February 2017 11:55
To: Clinical Trial Helpline
Subject: Re: SCOPE - Food allergy study

Sorry: very similar design: still 2 phases, but in first phase participants will receive either cow's milk (or Protifar, if we go down that route) or placebo (soya milk or alternative) while in 2nd phase all subjects will get cow's milk (supermarket-sourced).

Many thanks,

Paul Turner