BMJ Open

Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) A randomised, controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032844
Article Type:	Protocol
Date Submitted by the Author:	10-Jul-2019
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Keywords:	Immunology < BASIC SCIENCES, Eczema < DERMATOLOGY, INFECTIOUS DISEASES, Paediatric infectious disease & immunisation < PAEDIATRICS, Asthma < THORACIC MEDICINE, Allergy < THORACIC MEDICINE

Note from the Editors: Instructions for reviewers of study protocols

Since launching in 2011, BMJ Open has published study protocols for planned or ongoing research studies. If data collection is complete, we will not consider the manuscript.

Publishing study protocols enables researchers and funding bodies to stay up to date in their fields by providing exposure to research activity that may not otherwise be widely publicised. This can help prevent unnecessary duplication of work and will hopefully enable collaboration. Publishing protocols in full also makes available more information than is currently required by trial registries and increases transparency, making it easier for others (editors, reviewers and readers) to see and understand any deviations from the protocol that occur during the conduct of the study.

The scientific integrity and the credibility of the study data depend substantially on the study design and methodology, which is why the study protocol requires a thorough peer-review.

BMJ Open will consider for publication protocols for any study design, including observational studies and systematic reviews.

Some things to keep in mind when reviewing the study protocol:

- Protocol papers should report planned or ongoing studies. The dates of the study should be included in the manuscript.
- Unfortunately we are unable to customize the reviewer report form for study protocols. As such, some of the items (i.e., those pertaining to results) on the form should be scored as Not Applicable (N/A).
- While some baseline data can be presented, there should be no results or conclusions present in the study protocol.
- For studies that are ongoing, it is generally the case that very few changes can be made to the methodology. As such, requests for revisions are generally clarifications for the rationale or details relating to the methods. If there is a major flaw in the study that would prevent a sound interpretation of the data, we would expect the study protocol to be rejected.

Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) A randomised, controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

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ABSTRACT

Introduction Bacillus Calmette-Guérin (BCG) vaccination reduces all-cause infant mortality in high-mortality settings by more than can be attributed to protection against tuberculosis. This is proposed to result from non-specific protection against non-vaccine targeted ('off-target') infections. There is also evidence that BCG protects against allergic diseases.

Methods The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) is a phase III multicentre, single-blinded, randomised controlled trial. A total of 1438 healthy neonates will be randomised to receive either BCG vaccination or no BCG vaccination in the first 10 days of life. Measures of allergy, eczema, infection, and asthma will be obtained from parent-completed questionnaires 3 monthly in the first year and 6-monthly from one to five years of age, and clinical assessments at one and five years of age. Biological samples will also be collected for future immunological studies.

Analysis Primary outcome: The proportion of participants with measures of allergy and infection (atopic sensitisation, eczema, lower respiratory tract infection) at one and five years of age, and asthma at five years of age. Secondary outcomes: (1) the proportion of participants with additional measures of allergy, eczema, asthma and infections; (2) medication use for eczema and asthma; (3) the severity and age of onset of eczema and asthma; (4) the number of episodes of infection; (5) hospitalisations for infections; and (6) laboratory measures of immune responses.

Ethics and dissemination This trial has ethical and governance approval from Mercy Health Human Research Ethics Committee (HREC, No. R12-28) and Royal Children's Hospital HREC (No. 33025) with additional governance approval from Barwon Health and St John of God, Geelong, Victoria. Results of this trial will be published in peer-reviewed journals and presented at scientific conferences.

Trial Registration number: Clinical trials.gov NCT01906853

ARTICLE SUMMARY

Strengths and limitations of this study

- Infections remain a leading cause of mortality in infants and allergic diseases and asthma are increasing causes of infant/child morbidity
- Large-scale randomised trials for non-specific beneficial effects of neonatal BCG vaccination in high-income countries are lacking
- Neonatal BCG vaccination could be a safe and low-cost means to improve infant health
- No placebo was given to the control group because the development of a scar in BCG vaccinated infants prevents the blinding of parents to infants' randomisation group.
- BCG was followed by multiple administrations of non-live vaccines, and this might attenuate the beneficial non-specific effects of BCG

INTRODUCTION

The Bacillus Calmette-Guérin (BCG) vaccine is given to more than 85 percent of infants worldwide to protect against tuberculosis (TB).[1] In addition to protecting against TB, vaccination with BCG-Denmark reduces all-cause neonatal mortality in a high-mortality setting,[2-4] likely by protecting against non-mycobacterial infections.[4 5]

Observational studies suggest that the beneficial 'non-specific' (heterologous) effects of BCG on the developing immune system may also reduce the prevalence of allergic disease and asthma in children.[6] However, meta-analyses of observational studies have had inconsistent findings.[7-9] The two randomised controlled trials (RCTs) that have investigated the effect of BCG on infant allergic disease both found that BCG vaccination reduced infant eczema (medication for eczema at 18 months of age;[10] clinically diagnosed eczema at 13 months of age[11]) but did not have a statistically significant effect on the prevalence of allergic sensitisation or food allergy at 13 or 18 months of age. However, in these studies, allergic outcomes were determined by parent questionnaire and serum IgE, rather than clinical assessment. Studies assessing the impact of BCG vaccination on asthma have had more consistent findings with two meta-analyses concluding the BCG reduces the risk of asthma by 14-27%.[7 9]

As a result of a reduction in the prevalence of TB in several high- and middle-income countries, BCG has been removed from routine vaccination schedules.This might have contributed to the increased prevalence of allergic diseases over the past three decades.[12-16] It is proposed that, by acting as an early life microbial stimulus,[17] BCG prevents allergy by skewing the developing immune system in predisposed individuals away from the T helper (Th) 2 type immune response typically associated with allergy.[6 8]

BCG vaccination induces potent Th1 responses in neonates. In adults, it induces trained immunity in innate immune cells[18 19] and promotes Th1 and Th17 responses to non-mycobacterial pathogens.[20-22]

Two large observational studies provide further evidence that BCG vaccination protects against non-mycobacterial infections, particularly sepsis and respiratory infections.[23 24] However, BCG-mediated protection against these non-vaccine targeted or 'off-target' infections might be limited by subsequent vaccination with non-live vaccines which are proposed to counteract the beneficial non-specific effects of BCG.[4 25 26] Studies in a low-mortality setting, including a recent RCT of neonatal BCG vaccination,[27] and an observational study of over 19,000 infants,[28] support this hypothesis with protective effects of BCG against off-target infections most evident in early infancy, before administration of non-live vaccines.[29] Moreover, in the RCT, the protective effect of BCG was only evident in infants of BCG-vaccinated mothers.[27]

Subsequent to a 2004 WHO recommendation, an increasing number of countries have implemented routine neonatal hepatitis B vaccination.[30] Therefore, the potential impact of this vaccine on the beneficial non-specific effects of neonatal BCG vaccination also requires consideration.

Australia, where routine BCG vaccination was halted in the 1980s - has one of the highest rates of infant allergic disease globally.[31-33] It therefore represents an ideal setting in which to assess the impact of BCG on allergic disease. The Melbourne Infant Study: BCG for Allergy

and Infection Reduction (MIS BAIR) is an RCT to investigate whether neonatal BCG vaccination reduces the prevalence of allergic and infectious disease.

STUDY AIMS

Primary aims

To determine whether neonatal BCG vaccination compared with no BCG vaccination, reduces allergic disease, infection and asthma in infants and children in Australia.

Secondary aims

To evaluate the immunological mechanisms underlying the non-specific effects of BCG by comparing the immune responses in BCG-vaccinated to those in BCG-naïve infants.

METHODS AND ANALYSIS

Study design and setting

This is a phase III multicentre, single-blinded, RCT of neonatal BCG vaccination compared with no BCG vaccination. Clinical trials.gov NCT01906853. (Recruitment status: completed).

The study population will be healthy neonates 0 to 10 days of age born at one of the study site hospitals in Victoria, Australia. Recent studies of the burden of allergic diseases in this population have reported high a prevalence of eczema (28% [34]), allergic sensitisation (18%) and clinically significant food allergy (10.4% [35]) at 12 months of age, as well as high rates (22%) of asthma by 4-5 years.[36]

Study sites comprise: the Murdoch Children's Research Institute (MCRI), Melbourne; Royal Children's Hospital (RCH) Melbourne; Mercy Hospital for Women, Heidelberg; Werribee Mercy Hospital, Werribee; University Hospital Geelong, Geelong; and St John of God Geelong Hospital, Geelong.

Patient and Public Involvement

Patients and public were not involved in the design of this study. The results of this study will be disseminated to study participants via participant newsletter.

Eligibility criteria

The inclusion criteria comprise: healthy neonates up to 10 days of age; birth weight greater than 1500 grams; gestational age equal to or greater than 32 weeks, mothers testing HIV negative during pregnancy, English-speaking mother and parent/legal guardian able to complete questionnaires in English and attend study visit. The exclusion criteria comprise: any indication for BCG vaccination in the first year of life as per the Australian national guidelines [37]; known or suspected HIV infection; infant at risk of immunodeficiency; serious underlying illness (including fever) or medical instability; skin infection or other skin condition; need for treatment with hepatitis B immunoglobulin; multiple-birth of more than twins; older sibling in the study.

Intervention

Infants will be randomised to receive BCG vaccination or no BCG vaccination in the first 10 days of life. Infants randomised to the BCG vaccination group will be administered a single

intradermal injection of 0.05 mL *Mycobacterium bovis* BCG vaccine, Danish Strain 1331(1-4 x 10^5 colony forming units) over the left deltoid within 24 hours of randomisation.

Reasons for withdrawal

Reasons for withdrawal from the study will be recorded along with demographic data. These may include: protocol violations; indication for participant to receive BCG vaccine within the first year of life;[37] serious adverse event (SAE) or other adverse event (AE); or guardian/parent request.

Data and sample collection

Timing of data and sample collection in MIS BAIR is summarised in Table 1. Web-based questionnaires will be administered to parents at the time of recruitment, randomisation and when the participant is 3, 6, 9 and 12 months of age as well as 6-monthly up to 5 years of age using the Research Electronic Data Capture (REDCap) platform.[38] Infant perinatal data, including infections, medications and hospital admission will be obtained from the birth site records. Participants will be invited to optional study visits for biological sample collection 7 days (± 4 days) after randomisation and at 6 months (5-8 months) of age. Infants will be invited for clinical assessment and biological sample collection at 1 year (between 11 and 24 months) and 5 years (between 5 and 6 years) of age.

Table 1: Data and sample collection schedule

Infant age	Antenatal	0-10d	3m	6m	9m	1y	1.5y	2y	2.5y	3y	3.5y	4y	4.5y	5y
Recruitment & randomisation														
Eligibility check and consent	✓	✓												
Baseline questionnaire	✓	✓												
Birth questionnaire		✓												
Randomisation ± vaccination		✓												
Full blood examination		✓[a]												
Post randomisation														
Perinatal hospital data		✓												
Parent questionnaire			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clinical eczema assessment						✓								✓
Skin prick test						✓								✓
Oral food challenge						✓[b]								✓[b]
Biological sample collection		✓[c]		✓[d]		✓								✓

[a] soon after birth; [b] if indicated by skin prick test result; [c] 7±4 days post randomisation; [d] 5-8 months of age; d, days; m, months; y, year

Parent questionnaires

To collect data on potential confounding factors and for stratification prior to randomisation, baseline parent questionnaires will be used to collect data on demographic, environmental, prenatal, and birth factors (Table 2). When the participants are 3, 6, 9 and 12 months of age, questionnaires will be provided to parents to collect data on diet, medications and potential environmental confounders, and to collect data for primary and secondary outcomes (Table 3).

Table 2: Baseline questionnaire data

Family and demographic	Family history of allergic disease (allergy, eczema, hay fever, asthma), maternal BCG vaccination, parent education, parent country of birth, ethnicity.

Household environment	Size, composition, smoking, pets.
Maternal prenatal	Age, weight, height, prior pregnancies, vaccinations, smoking, antibiotics, vitamin D, probiotics other medications or supplements.
Birth	Mode of delivery*, birth site*, plurality*, birth complications, gestational age, weight, sex, antibiotics or other medications during labour.

* Required for stratification prior to randomisation

Table 3: Three- and six-monthly questionnaire outcome data

Illnesses	Any episode of illness, infant age, duration of illness, symptoms, medial consultations and hospital admissions, diagnosis and any treatments/medications.
Eczema	Symptoms of eczema, infant age at onset of eczema, distribution of eczema, use of eczema medications, medical consultations and hospital admissions. Includes modified U.K. working party's diagnostic criteria for atopic dermatitis and modified patient-oriented eczema measure (POEM) tool questions.[52 53]
Allergies	Any episodes of food or other allergies, infant age, allergen, symptoms, severity, diagnosis and any treatment.
Asthma	International study of asthma and allergies in childhood (ISAAC) questions,[54 55] asthma medication usage, symptom severity, acute exacerbations and health care utilisation, including hospitalisations
Diet	Breast milk feeding, formula milk feeding, food introduction, dietary supplements.
Other	Household composition, childcare, pets and other animals, household smoking, drinking water source, vaccinations, other medications or supplements, other diseases/disabilities, BCG complications, overseas travel, non-illness associated hospital admissions.

Clinical assessments

Allergic sensitisation: Skin prick testing (SPT) to the following panel of allergens will be assessed: food allergens - cow's milk, raw egg, peanut, sesame, cashew, hazelnut, shellfish, walnut (at 5-year visit only); other allergens - *Dermatophagoides pteronyssinus 1* (house dust mite), cat, dog, *Alternaria tenuis* (mould) and rye grass pollen. SPT will be done according to standard guidelines.[39] For each food allergen tested, data will also be collected on prior ingestion, exposure, reactions and tolerance (after the SPT wheal size is assessed).

Eczema: Severity of eczema will be assessed using Scoring of Atopic Dermatitis (SCORAD).[40]

Oral food challenge (OFC): Participants with a wheal diameter ≥1 mm greater than the negative control to selected food allergens during SPT will be invited to have an OFC as detailed in supplementary Figure 1. OFCs will be done at MCRI according to Australasian Society of Clinical Immunology and Allergy (ASCIA) guidelines as used by the RCH Allergy Clinic and the HealthNuts study.[41] The different pathways that will occur during an OFC detailed in supplementary Figure 2.

Other: Weight, height, skin, eye and hair colour, BCG vaccination site including scar measurement and photograph. BCG vaccination site assessment will occur after all other assessments are complete.

Biological sample collection

Full blood examination: A capillary blood sample will be collected from participants soon after birth as a preliminary screen for primary T-cell immunodeficiencies. This may be done before or after randomisation.

Peripheral blood: Peripheral blood samples will be collected at 7 days, 7 months, 1 year and 5 years of age. These samples will be used for immediate immune stimulation experiments as well as separation and storage of peripheral blood mononuclear cells and granulocytes, plasma and plasma-depleted cells at -80˚C or in liquid nitrogen, as appropriate. These samples will be retained for future immunological analysis.

Stool: From the day of birth participant's parents will be requested to collect a stool sample on each day (up to seven samples) and store it in a domestic freezer until collection at the 7-day post randomisation study visit. Additional stool samples will be collected during the 7-month and 1-year study visits or by parents prior to the one-year study visit using an in-home stool collection kit provided by the study team.

Outcomes

Primary outcomes

The primary outcomes are the proportions of infants with the following measures of allergic disease and infection at 1 year and 5 years of age:

- atopic sensitisation (positive SPT) to one or more of a panel of food and aeroallergens
- eczema
- lower respiratory tract infection (LRTI)
- asthma (5 years of age only)

Secondary outcomes

The secondary outcomes are the following additional measures of allergy and infection:

Allergy

The proportion of infants with clinical food allergy (Supplementary figures 1-2); atopic sensitisation with ≥ 3 mm wheal diameter; atopic sensitisation to multiple allergens; parent report of food allergy to any food; atopic sensitisation to egg allergen; atopic wheeze.

Eczema

Severity of eczema; age of onset of eczema; and proportions of clinically diagnosed eczema and steroid use for eczema.

Infections

Proportion of infants with or frequency of the following: any infection; upper respiratory tract infection; LRTI; diarrhoea with vomiting; rash with fever; fever; hospitalisation for infection; hospitalisation for respiratory tract infection.

Asthma,

The proportion of infants with current asthma, asthma severity and medication use for asthma

Sample size and power calculation

This study aims to randomise a total of 1,438 participants. This sample size is calculated based on an assumed minimum 80% retention rate, resulting in an expected minimum 1,150 infants with 1-year data available. With a final sample size of 575 in each arm, this study is powered to detect a minimum 35% reduction in atopic sensitisation, 25% reduction in eczema and 25% reduction in LRTI with a power of 80% in the first year of life and a minimum 37% reduction in atopic sensitisation, 26% reduction in eczema, 26% reduction in LRTI and 27% reduction in asthma with a power of 80% at five years of age. These differences are based on the previously reported prevalence of atopic sensitisation, eczema, LRTI and asthma in Australian infants at 1 and 5 years of age. [35 36 42]

Recruitment

Recruitment will occur in two stages: (1) early consent will be sought from pregnant women attending antenatal clinics or in the postnatal ward at a study site; (2) additional pregnant women or mothers interested in participating but not being cared for at a study site may also be recruited if they contact the research team antenatally or within 10 days of delivery. Consent to participate in the study will be verbally confirmed after the birth of antenatally recruited infants and prior to randomisation.

Randomisation

Recruited neonates will only be randomised after confirmation that they still fulfil inclusion criteria and do not meet any exclusion criteria. Randomisation to BCG-vaccination or no BCG vaccination will be done in a 1:1 ratio using the REDCap randomisation function.[38] The randomisation schedule will be established by a statistician external to the study using random permuted blocks with a minimum of three different block sizes. Randomisation will be stratified by: (i) site (hospital); (ii) method of delivery (Caesarean vs non-Caesarean); and (iii) plurality of birth (twins vs singletons). Twins will be assigned to the same intervention arm.

Blinding

As BCG vaccination results in the formation of a scar at the vaccination site in 93-99% of infants,[43-46] blinding with the use of a placebo is not possible. For blinding of clinical assessments, prior to commencement of the 1- and 5-year study visits, each participant's left upper arm will be covered with a bandage by the parent or a member of the study team not involved with the assessment to hide the potential scar site. Clinical assessments will be done by a member of the study team or clinical staff member who was not involved in the randomisation of the participant.

Statistical analysis

Statistical analysis of primary and secondary outcomes will be overseen by the trial statistician. Primary analysis will be by intention to treat, including all randomised participants where outcome data are available. Data will be collected according to Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting randomised trials.[47]

Outcomes

Primary outcomes

Comparison between BCG-vaccinated and BCG-naïve infants will be estimated using binary regression adjusted for the stratification factors used during randomisation. Results will be presented as risk difference with 95% confidence intervals (CI).

Secondary outcomes

Comparison between the BCG-vaccinated and BCG-naïve infants will be estimated using binary linear or Poisson regression adjusted for the stratification factors used during randomisation. Results will be presented as risk difference with 95% CI.

Missing data

If the proportion of missing data is less than 5%, the primary analysis will be a complete case analysis. Otherwise, the frequency and patterns of missing data will be examined and, if appropriate, multiple imputation models will be conducted for the outcome variables. Fifty completed data sets will be imputed by chained equations including all the children initially randomised. The primary outcome, strata variable (mode of birth: vaginal/caesarean), and the variables predictive of missingness and allergy, eczema, infection or asthma will be included in the imputation model.

Subgroup analysis

Prior to any subgroup analysis, adjusted models including the stratification factors used in randomisation, the randomisation assignment and the subgroup variable as covariates will be used to estimate the interaction between the intervention and the subgroup variable. Where these models provide evidence that the intervention varies between subgroups, specific subgroup estimates and confidence intervals will be presented obtained from the adjusted model. The subgroups are presence or absence of BCG scar; timing of BCG administration; maternal BCG vaccination; sex; mode of delivery; season of birth; timing of hepatitis B vaccination. For allergy, eczema and asthma outcomes, an additional subgroup variable, family history of allergic disease or asthma, will be assessed.

Data monitoring and auditing

The independent Data Safety and Monitoring Committee (DSMC), consisting of an independent statistician, neonatologist and paediatric infectious diseases consultant will meet to review data and participant safety 6-monthly.

Risks

The potential risks of participation in this study may be related to: (1) adverse reactions to BCG vaccination: subcutaneous abscess, exaggerated local reaction, lymphadenitis, keloid scaring, osteitis and disseminated infection.[48] In Australia, adverse reactions to BCG vaccine occur in 15.3 out of 10,000 doses;[49] (2) blood collection: discomfort, bruising and rarely minor infection or blood clots (3) clinical assessments of allergy: anaphylactic reactions may occur during OFC, during SPT (rare) and as a late reaction to OFC (from further allergen exposures during the subsequent week). The protocols and workflows for SPT and OFC mitigate the risk of anaphylactic reactions and their safety has been demonstrated in previous clinical trials (supplementary Figures 1 and 2).[41] All AE will be recorded, and SAEs reported to the study site HREC and the DSMC.

Outlook and significance

The findings of MIS BAIR will provide evidence as to whether neonatal BCG vaccination, a low-cost, readily available intervention, reduces the prevalence of allergies and infections in the first one and five years of life, and asthma in the first five years of life. An immune

priming benefit of neonatal BCG against these diseases would have considerable public health implications and thus inform guidelines for BCG vaccine policies worldwide.

Limitations

The potential limitations of MIS BAIR include the inability to blind the parent(s)/guardian(s) to the infant's randomisation assignment. This may lead to bias or lower compliance if parent(s)/guardian(s) are disappointed with the randomisation assignment. However, blinding will be done for the 13-month study clinical assessments from which several of the primary and secondary outcomes measures of allergy and eczema are obtained. We expect that there will be greater recruitment of participants with a family history of allergic disease, which may limit the generalisability of our findings. We will therefore collect data in relation to family history to enable us to consider for this in later analyses. BCG was followed by multiple administrations of non-live vaccines, and this might attenuate the beneficial non-specific effects of BCG.[50 51]

ETHICS AND DISSEMINATION

This trial has been approved by the Mercy Health Human Research Ethics Committee (HREC, No. R12-28) and RCH HREC (No. 33025) with governance approval from Barwon Health and St John of God, Geelong. A record of consents and refusals will be maintained on the study REDCap database. Parent(s)/guardian(s) of participants will be informed of their option to withdraw from the study at any time. An electronic or hard copy parent /guardian information and consent form (PGICF) will be completed a parent/guardian as part of the consent process. Results of this trial will be published in peer-reviewed journals and presented at scientific conferences.

Contributors NC is the lead investigator and responsible for study conception, design and funding acquisition. KG developed the trial database. KG, NC, BF, SD, LC, CZ developed the ethics application and all other authors provided critical evaluation and revision. DC, KG, PV, CM, VA, NC developed the recruitment methods. KG, BF, VA, ALP, KA, PV developed and SD, MS, NC contributed to the design of the questionnaires. KG, KA developed and ALP, NC, PV contributed to the clinical assessment methods. CZ, NC, BF, SG, KF developed and KG, NM, RRB, PV contributed to biological sample collection and processing methods. SD developed the statistical analysis plan. NC, KG, KA, FS, VA, NM, MS contributed to and ALP, KF, RRB, PV, DC provided critical evaluation and revision. NM drafted the manuscript, co-ordinated manuscript preparation and revision. All authors provided critical evaluation and revision of the manuscript, and have approved the final version of this manuscript.

Funding This study is funded by research grants from the National Health and Medical Research Council (NHMRC) of Australia, The University of Melbourne, RCH foundation and MCRI.
Disclaimer The study funders did not have authority over the study design or preparation of this report.
Competing Interests KA has received speaker's honoraria from Abbott, Danone, Nestle and Alphapharm. All other authors have no competing interests to declare.
Patient consent Parent/guardian consent obtained
Ethics approval Mercy Health HREC and RCH HREC
Provenance and peer review Not commissioned; externally peer reviewed.

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Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) A randomised, controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

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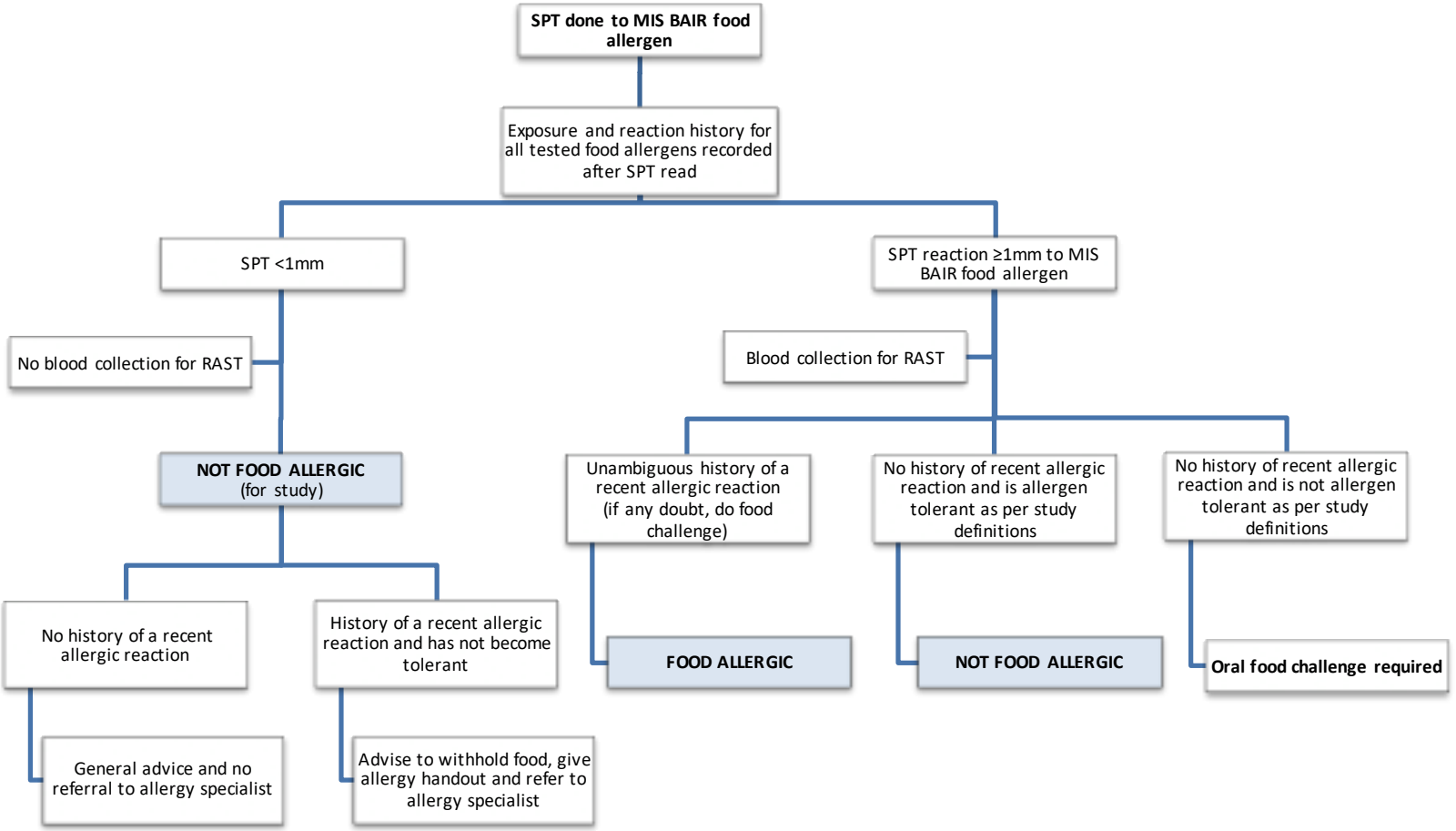
Supplementary Figures



Total dose of allergen as per food challenge protocol

- Cows milk: 160 ml
- Raw egg white: 1x 60 g egg
- Sesame: 2 teaspoons
- Peanut: 2 teaspoons
- Cashew: 2 teaspoons
- Hazelnut: 2 teaspoons
- Shellfish: 1 peeled prawn (~15 g)

Definitions

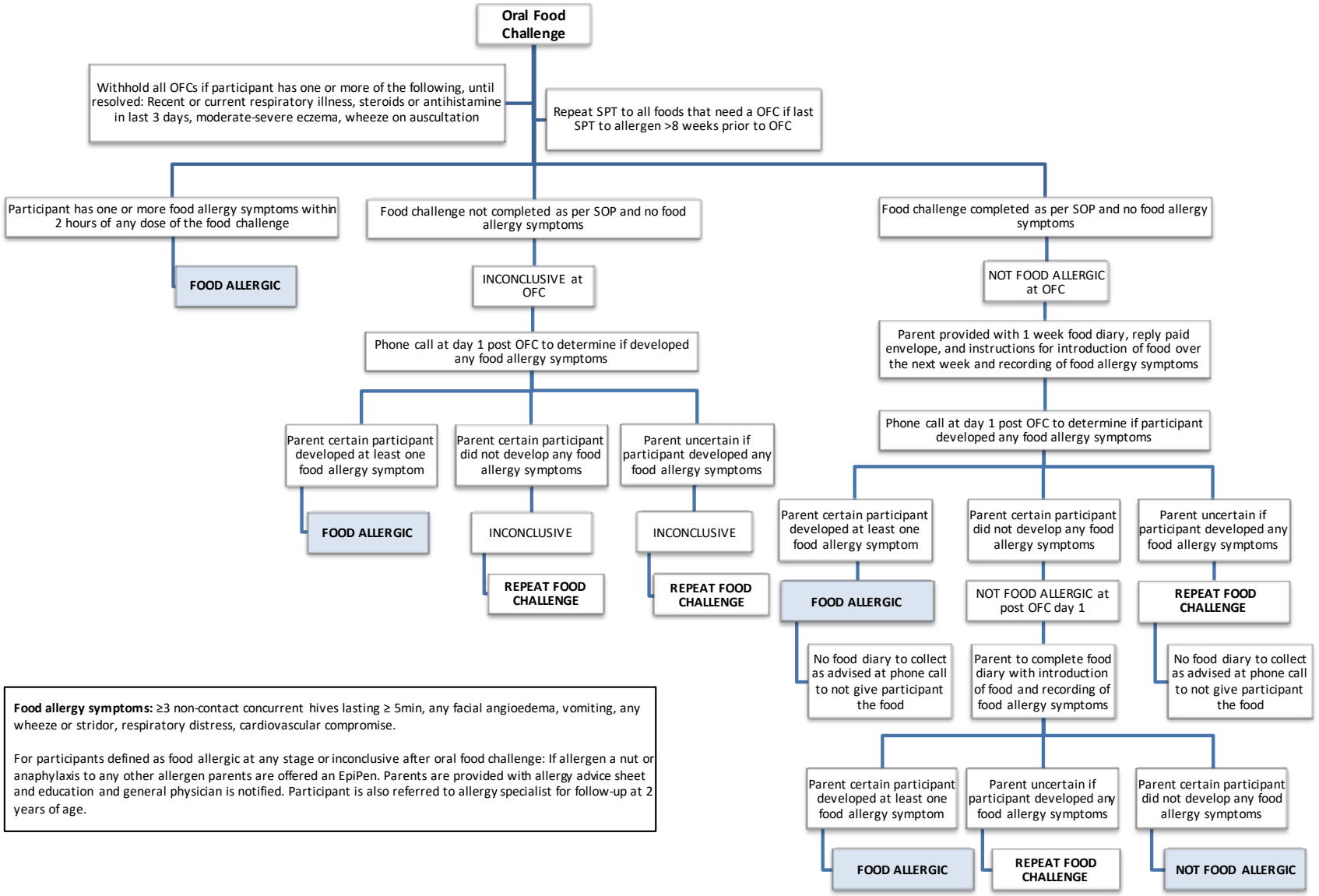
History of a recent allergic reaction: In the past 1-2 months (1 month for egg or milk, 2 months for any other allergen), at least one of the following reactions within 30 minutes of exposure to the MIS BAIR food allergen: ≥3 non-contact concurrent hives lasting ≥5 mins, vomiting (not gagging), facial angioedema, wheeze or stridor, respiratory distress, cardiovascular compromise (pale and floppy infant, cardiac arrest).

Allergen tolerance: In the past 1-2 months (1 month for egg or milk, 2 months for any other allergen), infant has eaten equivalent to ≥ 'total dose of food challenge protocol' of allergen on 2 separate occasions without reaction.

Adequate exposure: Food eaten not just touched and food in form that is recognizable (e.g. not mixed nuts) unless infant has previously tolerated all the other food in the dish.

Supplementary Figure 1: MIS BAIR skin prick test flow chart

SPT skin prick test, RAST radioallergosorbent test



Supplementary Figure 2: MIS BAIR oral food challenge flow chart

OFC oral food challenge, SPT skin prick test, SOP standard operating procedure

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____2 & 4___
	2b	All items from the World Health Organization Trial Registration Data Set	Available at Clinical trials.gov
Protocol version	3	Date and version identifier	Approved HREC application
Funding	4	Sources and types of financial, material, and other support	_____11_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	____1 & 11___
	5b	Name and contact information for the trial sponsor	_____1_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____11_____

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
	6b	Explanation for choice of comparators	3
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	___7-8_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	____5_____
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___8_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___8_____

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___8_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	___8_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	___8_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___9_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___9_____

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	__5-7_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___9_____
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Approved HREC application
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9 & clinical trials.gov
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___9_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	____9_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	____10____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	___NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___10_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	___10_____
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___10_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	___NA_____
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Approved HREC application
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Approved HREC application
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	5 & Approved HREC application
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	__11_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Approved HREC application
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Approved HREC application
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	__11_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	__NA____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	__NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Approved HREC application

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7 & Approved HREC application

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

BMJ Open

Study protocol for the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR), a randomised controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032844.R1
Article Type:	Protocol
Date Submitted by the Author:	31-Oct-2019
Complete List of Authors:	Messina, Nicole; Murdoch Childrens Research Institute, Infectious Diseases; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics Gardiner, Kaya; Murdoch Childrens Research Institute, Infectious Diseases Donath, Susan; Murdoch Childrens Research Institute, Clinical Epidemiology and Biostatistics Unit; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics Flanagan, Katie; University of Tasmania, School of Health Sciences; RMIT University, School of Health and Biomedical Science Ponsonby, Anne-Louise; Murdoch Childrens Research Institute, Center for Food and Allergy Research; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics Shann, Frank; Royal Children's Hospital Melbourne, Intensive Care Unit; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics RobinsBrowne, Roy; University of Melbourne, Microbiology and Immunology; Murdoch Childrens Research Institute, Infectious Diseases Freyne, Bridget; Murdoch Childrens Research Institute, Infectious Diseases; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics Abruzzo, Veronica; Murdoch Childrens Research Institute, Infectious Diseases Morison, Clare; Murdoch Childrens Research Institute, Infectious Diseases Cox, Lianne; Murdoch Childrens Research Institute, Infectious Diseases; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics Germano, Susie; Murdoch Childrens Research Institute, Infectious Diseases Zufferey, Christel; Murdoch Childrens Research Institute, Infectious Diseases Zimmermann, Petra; Murdoch Childrens Research Institute, Infectious Diseases; The University of Melbourne Faculty of Medicine Dentistry and Health Sciences, Department of Paediatrics Allen, Katie; Formerly of Murdoch Children's Research Institute, Centre for Food and Allergy Research Vuillermin, P; Deakin University School of Medicine; Barwon Health,

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Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Immunology (including allergy), Infectious diseases, Paediatrics
Keywords:	Immunology < BASIC SCIENCES, Eczema < DERMATOLOGY, INFECTIOUS DISEASES, Paediatric infectious disease & immunisation < PAEDIATRICS, Asthma < THORACIC MEDICINE, Allergy < THORACIC MEDICINE

Study protocol for the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR), a randomised controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

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ABSTRACT

Introduction: Bacillus Calmette-Guérin (BCG) vaccination reduces all-cause infant mortality in high-mortality settings by more than can be attributed to protection against tuberculosis. This is proposed to result from non-specific protection against non-vaccine targeted ('off-target') infections. There is also evidence that BCG protects against allergic diseases.

Methods and analysis: The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) is a phase III multicentre, single-blinded, randomised controlled trial. A total of 1438 healthy neonates will be randomised to receive either BCG vaccination or no BCG vaccination in the first 10 days of life. Measures of allergy, eczema, infection, and asthma will be obtained from parent-completed questionnaires 3 monthly in the first year and 6-monthly from one to five years of age, and clinical assessments at one and five years of age. Biological samples will also be collected for future immunological studies.

Analysis Primary outcome: The proportion of participants with measures of allergy and infection (atopic sensitisation, eczema, lower respiratory tract infection) at one and five years of age, and asthma at five years of age. Secondary outcomes: (1) the proportion of participants with additional measures of allergy, eczema, asthma and infections; (2) medication use for eczema and asthma; (3) the severity and age of onset of eczema and asthma; (4) the number of episodes of infection; (5) hospitalisations for infections; and (6) laboratory measures of immune responses.

Ethics and dissemination: This trial has ethical and governance approval from Mercy Health Human Research Ethics Committee (HREC, No. R12-28) and Royal Children's Hospital HREC (No. 33025) with additional governance approval from Barwon Health and St John of God, Geelong, Victoria. Results of this trial will be published in peer-reviewed journals and presented at scientific conferences.

Trial Registration number: Clinical trials.gov NCT01906853

ARTICLE SUMMARY

Strengths and limitations of this study

- The use of well-defined, internationally accepted outcome measures in a large-scale randomised trial
- Low TB burden in Australia reduces potential influence of *M. tuberculosis* exposure
- Use of oral food challenge in addition to skin prick testing provides a robust and clinically relevant measure of food allergy
- Inability to blind parents to infants' randomisation group due to the scar resulting from BCG vaccination
- Routine scheduled non-live vaccines administered subsequent to randomisation might attenuate the beneficial non-specific effects of BCG

INTRODUCTION

The Bacillus Calmette-Guérin (BCG) vaccine is given to more than 85 percent of infants worldwide to protect against tuberculosis (TB).[1] In addition to protecting against TB, vaccination with BCG-Denmark reduces all-cause neonatal mortality in a high-mortality setting,[2-4] likely by protecting against non-mycobacterial infections.[4 5]

Observational studies suggest that the beneficial 'non-specific' (heterologous) effects of BCG on the developing immune system may also reduce the prevalence of allergic disease and asthma in children.[6] However, meta-analyses of observational studies have had inconsistent findings.[7-9] The two randomised controlled trials (RCTs) that have investigated the effect of BCG on infant allergic disease both found that BCG vaccination reduced infant eczema (medication for eczema at 18 months of age;[10] clinically diagnosed eczema at 13 months of age[11]) but did not have a statistically significant effect on the prevalence of allergic sensitisation or food allergy at 13 or 18 months of age. However, in these studies, allergic outcomes were determined by parent questionnaire and serum IgE, rather than clinical assessment. Studies assessing the impact of BCG vaccination on asthma have had more consistent findings with two meta-analyses concluding the BCG reduces the risk of asthma by 14-27%.[7 9]

As a result of a reduction in the prevalence of TB in several high- and middle-income countries, BCG has been removed from routine vaccination schedules. This might have contributed to the increased prevalence of allergic diseases over the past three decades.[12-16] It is proposed that, by acting as an early life microbial stimulus,[17] BCG prevents allergy by skewing the developing immune system in predisposed individuals away from the T helper (Th) 2 type immune response typically associated with allergy.[6 8]

BCG vaccination induces potent Th1 responses in neonates. In adults, it induces trained immunity in innate immune cells[18 19] and promotes Th1 and Th17 responses to non-mycobacterial pathogens.[20-22]

Two large observational studies provide further evidence that BCG vaccination protects against non-mycobacterial infections, particularly sepsis and respiratory infections.[23 24] However, BCG-mediated protection against these non-vaccine targeted or 'off-target' infections might be limited by subsequent vaccination with non-live vaccines which are proposed to counteract the beneficial non-specific effects of BCG.[4 25 26] Studies in a low-mortality setting, including a recent RCT of neonatal BCG vaccination,[27] and an observational study of over 19,000 infants,[28] support this hypothesis with protective effects of BCG against off-target infections most evident in early infancy, before administration of non-live vaccines.[29] Moreover, in the RCT, the protective effect of BCG was only evident in infants of BCG-vaccinated mothers.[27]

Subsequent to a 2004 WHO recommendation, an increasing number of countries have implemented routine neonatal hepatitis B vaccination.[30] Therefore, the potential impact of this vaccine on the beneficial non-specific effects of neonatal BCG vaccination also requires consideration.

Australia, where routine BCG vaccination was halted in the 1980s - has one of the highest rates of infant allergic disease globally.[31-33] It therefore represents an ideal setting in which to assess the impact of BCG on allergic disease. The Melbourne Infant Study: BCG for Allergy

and Infection Reduction (MIS BAIR) is an RCT to investigate whether neonatal BCG vaccination reduces the prevalence of allergic and infectious disease.

STUDY AIMS

Primary aims

To determine whether neonatal BCG vaccination compared with no BCG vaccination, reduces allergic disease, infection and asthma in infants and children in Australia.

Secondary aims

To evaluate the immunological mechanisms underlying the non-specific effects of BCG by comparing the immune responses in BCG-vaccinated to those in BCG-naïve infants.

METHODS AND ANALYSIS

Study design and setting

This is a phase III multicentre, single-blinded, RCT of neonatal BCG vaccination compared with no BCG vaccination. The study was prospectively registered in clinical trials.gov NCT01906853 (Recruitment status: completed). Recruitment commenced in August 2013 and study follow-up will be completed by 2022.

The study population will be healthy neonates 0 to 10 days of age born at one of the study site hospitals in Victoria, Australia. Recent studies of the burden of allergic diseases in this population have reported high a prevalence of eczema (28% [34]), allergic sensitisation (18%) and clinically significant food allergy (10.4% [35]) at 12 months of age, as well as high rates (22%) of asthma by 4-5 years.[36]

Study sites comprise: the Murdoch Children's Research Institute (MCRI), Melbourne; Royal Children's Hospital (RCH) Melbourne; Mercy Hospital for Women, Heidelberg; Werribee Mercy Hospital, Werribee; University Hospital Geelong, Geelong; and St John of God Geelong Hospital, Geelong.

Patient and Public Involvement

Patients and public were not involved in the design of this study. The results of this study will be disseminated to study participants via participant newsletter.

Eligibility criteria

The inclusion criteria comprise: healthy neonates up to 10 days of age; birth weight greater than 1500 grams; gestational age equal to or greater than 32 weeks, mothers testing HIV negative during pregnancy, English-speaking mother and parent/legal guardian able to complete questionnaires in English and attend study visit. The exclusion criteria comprise: any indication for BCG vaccination in the first year of life as per the Australian national guidelines [37]; known or suspected HIV infection; infant at risk of immunodeficiency; serious underlying illness (including fever) or medical instability; skin infection or other skin condition; need for treatment with hepatitis B immunoglobulin; multiple-birth of more than twins; older sibling in the study.

Intervention

Infants will be randomised to receive BCG vaccination or no BCG vaccination in the first 10 days of life. Infants randomised to the BCG vaccination group will be administered a single

intradermal injection of 0.05 mL *Mycobacterium bovis* BCG vaccine, Danish Strain 1331(1-4 x 10^5 colony forming units) over the left deltoid within 24 hours of randomisation.

Reasons for withdrawal

Reasons for withdrawal from the study will be recorded along with demographic data. These may include: protocol violations; indication for participant to receive BCG vaccine within the first year of life;[37] serious adverse event (SAE) or other adverse event (AE); or guardian/parent request.

Data and sample collection

Timing of data and sample collection in MIS BAIR is summarised in Table 1. Web-based questionnaires will be administered to parents at the time of recruitment, randomisation and when the participant is 3, 6, 9 and 12 months of age as well as 6-monthly up to 5 years of age using the Research Electronic Data Capture (REDCap) platform.[38] Infant perinatal data, including infections, medications and hospital admission will be obtained from the birth site records. Participants will be invited to optional study visits for biological sample collection 7 days (± 4 days) after randomisation and at 6 months (5-8 months) of age. Infants will be invited for clinical assessment and biological sample collection at 1 year (between 11 and 24 months) and 5 years (between 5 and 6 years) of age.

Table 1: Data and sample collection schedule

Infant age	Antenatal	0-10d	3m	6m	9m	1y	1.5y	2y	2.5y	3y	3.5y	4y	4.5y	5y
Recruitment & randomisation														
Eligibility check and consent	✓	✓												
Baseline questionnaire	✓	✓												
Birth questionnaire		✓												
Randomisation ± vaccination		✓												
Full blood examination		✓[a]												
Post randomisation														
Perinatal hospital data		✓												
Parent questionnaire			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clinical eczema assessment						✓								✓
Skin prick test						✓								✓
Oral food challenge						✓[b]								✓[b]
Biological sample collection		✓[c]		✓[d]		✓								✓

[a] soon after birth; [b] if indicated by skin prick test result; [c] 7±4 days post randomisation; [d] 5-8 months of age; d, days; m, months; y, year

Parent questionnaires

To collect data on potential confounding factors and for stratification prior to randomisation, baseline parent questionnaires will be used to collect data on demographic, environmental, prenatal, and birth factors (Table 2). When the participants are 3, 6, 9 and 12 months of age, questionnaires will be provided to parents to collect data on diet, medications and potential environmental confounders, and to collect data for primary and secondary outcomes (Table 3).

Table 2: Baseline questionnaire data

Family and demographic	Family history of allergic disease (allergy, eczema, hay fever, asthma), maternal BCG vaccination, parent education, parent country of birth, ethnicity.

Household environment	Size, composition, smoking, pets, region (post code).
Maternal prenatal	Age, weight, height, prior pregnancies, vaccinations, smoking, antibiotics, vitamin D, probiotics other medications or supplements.
Birth	Mode of delivery*, birth site*, plurality*, birth complications, gestational age, weight, sex, antibiotics or other medications during labour.

* Required for stratification prior to randomisation

Table 3: Three- and six-monthly questionnaire outcome data

Illnesses	Any episode of illness, infant age, duration of illness, symptoms, medial consultations and hospital admissions, diagnosis and any treatments/medications.
Eczema	Symptoms of eczema, infant age at onset of eczema, distribution of eczema, use of eczema medications, medical consultations and hospital admissions. Includes modified U.K. working party's diagnostic criteria for atopic dermatitis and modified patient-oriented eczema measure (POEM) tool questions.[39 40]
Allergies	Any episodes of food or other allergies, infant age, allergen, symptoms, severity, diagnosis and any treatment.
Asthma	International study of asthma and allergies in childhood (ISAAC) questions,[41 42] asthma medication usage, symptom severity, acute exacerbations and health care utilisation, including hospitalisations
Diet	Breast milk feeding, formula milk feeding, food introduction, dietary supplements.
Other	Household composition, childcare, pets and other animals, household smoking, drinking water source, vaccinations, other medications or supplements, other diseases/disabilities, BCG complications, overseas travel, non-illness associated hospital admissions.

Clinical assessments

Allergic sensitisation: Skin prick testing (SPT) to the following panel of allergens will be assessed: food allergens - cow's milk, raw egg, peanut, sesame, cashew, hazelnut, shellfish, walnut (at 5-year visit only); other allergens - *Dermatophagoides pteronyssinus 1* (house dust mite), cat, dog, *Alternaria tenuis* (mould) and rye grass pollen. SPT will be done according to standard guidelines.[43] For each food allergen tested, data will also be collected on prior ingestion, exposure, reactions and tolerance (after the SPT wheal size is assessed).

Eczema: Severity of eczema will be assessed using Scoring of Atopic Dermatitis (SCORAD).[44]

Oral food challenge (OFC): Participants with a wheal diameter ≥1 mm greater than the negative control to selected food allergens during SPT will be invited to have an OFC as detailed in supplementary Figure 1. OFCs will be done at MCRI according to Australasian Society of Clinical Immunology and Allergy (ASCIA) guidelines as used by the RCH Allergy Clinic and the HealthNuts study.[45] The different pathways that will occur during an OFC detailed in supplementary Figure 2.

Other: Weight, height, skin, eye and hair colour, BCG vaccination site including scar measurement and photograph. BCG vaccination site assessment will occur after all other assessments are complete.

Biological sample collection

Full blood examination: A capillary blood sample will be collected from participants soon after birth as a preliminary screen for primary T-cell immunodeficiencies. This may be done before or after randomisation.

Peripheral blood: Peripheral blood samples will be collected at 7 days, 7 months, 1 year and 5 years of age. These samples will be used for immediate immune stimulation experiments as well as separation and storage of peripheral blood mononuclear cells and granulocytes, plasma and plasma-depleted cells at -80˚C or in liquid nitrogen, as appropriate. These samples will be retained for future immunological analysis.

Stool: From the day of birth participant's parents will be requested to collect a stool sample on each day (up to seven samples) and store it in a domestic freezer until collection at the 7-day post randomisation study visit. Additional stool samples will be collected during the 7-month and 1-year study visits or by parents prior to the one-year study visit using an in-home stool collection kit provided by the study team.

Outcomes

Primary outcomes

The primary outcomes are the proportions of infants with the following measures of allergic disease and infection at 1 year and 5 years of age:

- atopic sensitisation (positive SPT) to one or more of a panel of food and aeroallergens
- eczema (Williams' UK diagnostic criteria)
- lower respiratory tract infection (LRTI) episode ever (1 year) or hospitalisation ever (5 years)
- asthma (ISAAC definition) ever and current (5 years of age only)

Secondary outcomes

The secondary outcomes are the following additional measures of allergy and infection:

Allergy

The proportion of infants with clinical food allergy (Supplementary figures 1-2); atopic sensitisation with ≥ 3 mm wheal diameter to any allergen; atopic sensitisation to multiple allergens; parent report of food allergy to any food; atopic sensitisation to egg allergen; clinical egg allergy; atopic wheeze.

Eczema

Severity of eczema; age of onset of eczema; and proportions of clinically diagnosed eczema and steroid use for eczema.

Infections

Proportion of infants with or rate of the following: any infection; upper respiratory tract infection; LRTI; diarrhoea with vomiting; rash with fever; fever; hospitalisation for infection; hospitalisation for respiratory tract infection.

Asthma

The proportion of infants with current asthma, asthma severity and medication use for asthma

Sample size and power calculation

This study aims to randomise a total of 1,438 participants. This sample size is calculated based on an assumed minimum 80% retention rate, resulting in an expected minimum 1,150 infants with 1-year data available. With a final sample size of 575 in each arm, this study is powered to detect a minimum 35% reduction in atopic sensitisation, 25% reduction in eczema and 25% reduction in LRTI with a power of 80% in the first year of life and a minimum 37% reduction in atopic sensitisation, 26% reduction in eczema, 26% reduction in LRTI and 27% reduction in asthma with a power of 80% at five years of age. These differences are based on the previously reported prevalence of atopic sensitisation, eczema, LRTI and asthma in Australian infants at 1 and 5 years of age.[35 36 46]

Recruitment

Recruitment will occur in two stages: (1) early consent will be sought from pregnant women attending antenatal clinics or in the postnatal ward at a study site; (2) additional pregnant women or mothers interested in participating but not being cared for at a study site may also be recruited if they contact the research team antenatally or within 10 days of delivery. Consent to participate in the study will be verbally confirmed after the birth of antenatally recruited infants and prior to randomisation.

Randomisation

Recruited neonates will only be randomised after confirmation that they still fulfil inclusion criteria and do not meet any exclusion criteria. Randomisation to BCG-vaccination or no BCG vaccination will be done in a 1:1 ratio using the REDCap randomisation function.[38] The randomisation schedule will be established by a statistician external to the study using random permuted blocks with a minimum of three different block sizes. Randomisation will be stratified by: (i) site (hospital); (ii) method of delivery (Caesarean vs non-Caesarean); and (iii) plurality of birth (twins vs singletons). Twins will be assigned to the same intervention arm.

Blinding

As BCG vaccination results in the formation of a scar at the vaccination site in 93-99% of infants,[47-50] blinding with the use of a placebo is not possible. For blinding of clinical assessments, prior to commencement of the 1- and 5-year study visits, each participant's left upper arm will be covered with a bandage by the parent or a member of the study team not involved with the assessment to hide the potential scar site. Clinical assessments will be done by a member of the study team or clinical staff member who was not involved in the randomisation of the participant.

Statistical analysis

Statistical analysis of primary and secondary outcomes will be overseen by the trial statistician. Primary analysis will be by intention to treat, including all randomised participants where outcome data are available. Data will be collected according to Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting randomised trials.[51]

Outcomes

Primary outcomes

Comparison between BCG-vaccinated and BCG-naïve infants will be estimated using binary regression adjusted for the stratification factors used during randomisation. Results will be presented as risk difference with 95% confidence intervals (CI).

Secondary outcomes

Comparison between the BCG-vaccinated and BCG-naïve infants will be estimated using binary linear or Poisson regression adjusted for the stratification factors used during randomisation. Results will be presented as risk difference with 95% CI.

Missing data

If the proportion of missing data is less than 5%, the primary analysis will be a complete case analysis. Otherwise, the frequency and patterns of missing data will be examined and, if appropriate, multiple imputation models will be conducted for the outcome variables. Fifty completed data sets will be imputed by chained equations including all the children initially randomised. The primary outcome, strata variable (mode of birth: vaginal/caesarean), and the variables predictive of missingness and allergy, eczema, infection or asthma will be included in the imputation model.

Subgroup analysis

Prior to any subgroup analysis, adjusted models including the stratification factors used in randomisation, the randomisation assignment and the subgroup variable as covariates will be used to estimate the interaction between the intervention and the subgroup variable. Where these models provide evidence that the intervention varies between subgroups, specific subgroup estimates and confidence intervals will be presented obtained from the adjusted model. The subgroups are presence or absence of BCG scar; timing of BCG administration; maternal BCG vaccination; sex; mode of delivery; season of birth; timing of hepatitis B vaccination. For allergy, eczema and asthma outcomes, an additional subgroup variable, family history of allergic disease or asthma, will be assessed.

Data monitoring and auditing

The independent Data Safety and Monitoring Committee (DSMC), consisting of an independent statistician, neonatologist and paediatric infectious diseases consultant will meet to review data and participant safety 6-monthly.

Risks

The potential risks of participation in this study may be related to: (1) adverse reactions to BCG vaccination: subcutaneous abscess, exaggerated local reaction, lymphadenitis, keloid scaring, osteitis and disseminated infection.[52] In Australia, adverse reactions to BCG vaccine occur in 15.3 out of 10,000 doses;[53] (2) blood collection: discomfort, bruising and rarely minor infection or blood clots (3) clinical assessments of allergy: anaphylactic reactions may occur during OFC, during SPT (rare) and as a late reaction to OFC (from further allergen exposures during the subsequent week). The protocols and workflows for SPT and OFC mitigate the risk of anaphylactic reactions and their safety has been demonstrated in previous clinical trials (supplementary Figures 1 and 2).[45] All AE will be recorded, and SAEs reported to the study site HREC and the DSMC.

Outlook and significance

The findings of MIS BAIR will provide evidence as to whether neonatal BCG vaccination, a low-cost, readily available intervention, reduces the prevalence of allergies and infections in the first one and five years of life, and asthma in the first five years of life. An immune

priming benefit of neonatal BCG against these diseases would have considerable public health implications and thus inform guidelines for BCG vaccine policies worldwide.

Limitations

The potential limitations of MIS BAIR include the inability to blind the parent(s)/guardian(s) to the infant's randomisation assignment. This may lead to bias or lower compliance if parent(s)/guardian(s) are disappointed with the randomisation assignment. However, blinding will be done for the 13-month study clinical assessments from which several of the primary and secondary outcomes measures of allergy and eczema are obtained. We expect that there will be greater recruitment of participants with a family history of allergic disease, which may limit the generalisability of our findings. We will therefore collect data in relation to family history to enable us to consider for this in later analyses. BCG was followed by multiple administrations of non-live vaccines, and this might attenuate the beneficial non-specific effects of BCG.[54 55]

ETHICS AND DISSEMINATION

This trial has been approved by the Mercy Health Human Research Ethics Committee (HREC, No. R12-28) and RCH HREC (No. 33025) with governance approval from Barwon Health and St John of God, Geelong. A record of consents and refusals will be maintained on the study REDCap database. Parent(s)/guardian(s) of participants will be informed of their option to withdraw from the study at any time. An electronic or hard copy parent /guardian information and consent form (PGICF) will be completed a parent/guardian as part of the consent process. Results of this trial will be published in peer-reviewed journals and presented at scientific conferences.

Contributors NC is the lead investigator and responsible for study conception, design and funding acquisition. KG developed the trial database. KG, NC, BF, SD, LC, CZ developed the ethics application and all other authors provided critical evaluation and revision. DC, KG, PV, CM, VA, NC developed the recruitment methods. KG, BF, VA, ALP, KA, PV developed and SD, MS, NC contributed to the design of the questionnaires. KG, KA developed and ALP, NC, PV contributed to the clinical assessment methods. CZ, NC, BF, SG, KF developed and KG, NM, RRB, PV contributed to biological sample collection and processing methods. SD developed the statistical analysis plan. NC, KG, KA, FS, VA, NM, MS contributed to and ALP, KF, RRB, PV, DC provided critical evaluation and revision. NM drafted the manuscript, co-ordinated manuscript preparation and revision. PZ contributed to an early draft of the manuscript. All authors provided critical evaluation and revision of the manuscript, and have approved the final version of this manuscript.

Funding This study is funded by research grants from the National Health and Medical Research Council (NHMRC) of Australia, The University of Melbourne, RCH foundation and MCRI.
Disclaimer The study funders did not have authority over the study design or preparation of this report.
Competing Interests All other authors have no competing interests to declare.
Patient consent Parent/guardian consent obtained
Ethics approval Mercy Health HREC and RCH HREC
Provenance and peer review Not commissioned; externally peer reviewed.

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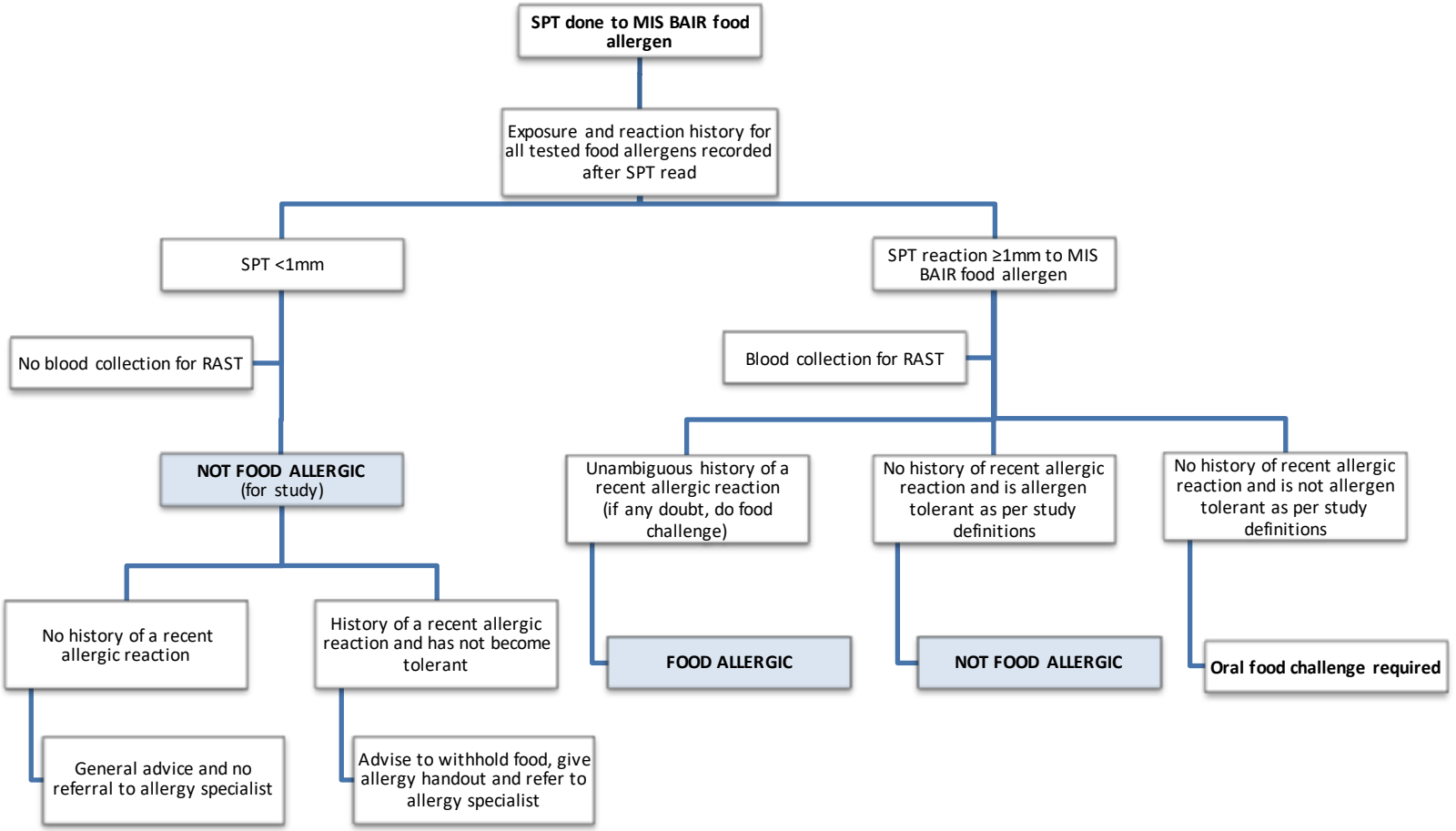
Study protocol for the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR), a randomised controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

*NL Messina, *K Gardiner, S Donath, K Flanagan, AL Ponsonby, F Shann, R Robins-Browne, B Freyne, V Abruzzo, C Morison, L Cox, S Germano, C Zufferey, P Zimmermann , KJ Allen, P Vuillermin, M South, D Casalaz, N Curtis

Supplementary Figures



Total dose of allergen as per food challenge protocol

- Cows milk: 160 ml
- Raw egg white: 1x 60 g egg
- Sesame: 2 teaspoons
- Peanut: 2 teaspoons
- Cashew: 2 teaspoons
- Hazelnut: 2 teaspoons
- Shellfish: 1 peeled prawn (~15 g)

Definitions

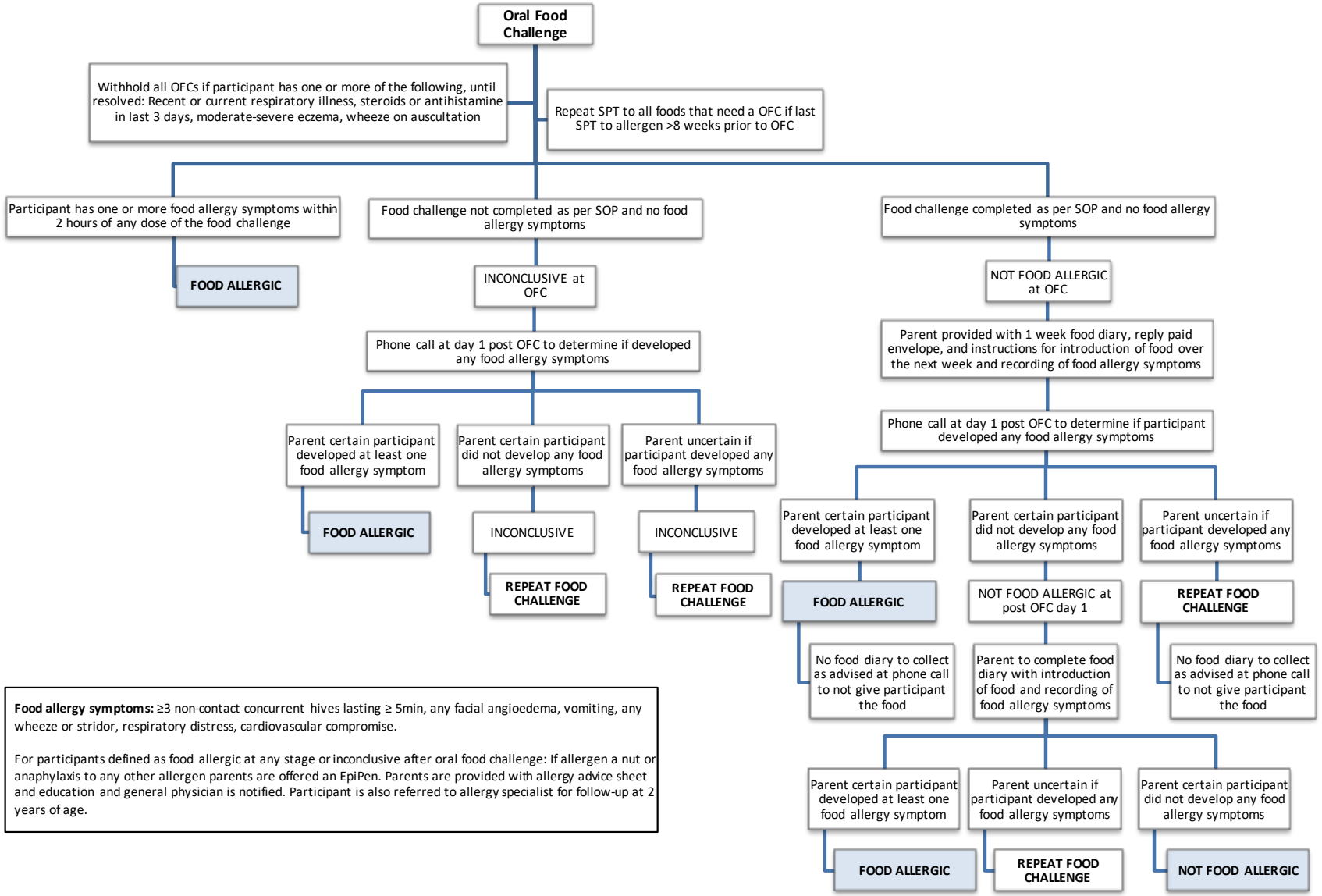
History of a recent allergic reaction: In the past 1-2 months (1 month for egg or milk, 2 months for any other allergen), at least one of the following reactions within 30 minutes of exposure to the MIS BAIR food allergen: ≥3 non-contact concurrent hives lasting ≥5 mins, vomiting (not gagging), facial angioedema, wheeze or stridor, respiratory distress, cardiovascular compromise (pale and floppy infant, cardiac arrest).

Allergen tolerance: In the past 1-2 months (1 month for egg or milk, 2 months for any other allergen), infant has eaten equivalent to ≥ 'total dose of food challenge protocol' of allergen on 2 separate occasions without reaction.

Adequate exposure: Food eaten not just touched and food in form that is recognizable (e.g. not mixed nuts) unless infant has previously tolerated all the other food in the dish.

Supplementary Figure 1: MIS BAIR skin prick test flow chart

SPT skin prick test, RAST radioallergosorbent test



Supplementary Figure 2: MIS BAIR oral food challenge flow chart

OFC oral food challenge, SPT skin prick test, SOP standard operating procedure

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____2 & 4___
	2b	All items from the World Health Organization Trial Registration Data Set	Available at Clinical trials.gov
Protocol version	3	Date and version identifier	Approved HREC application
Funding	4	Sources and types of financial, material, and other support	_____11_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	____1 & 11___
	5b	Name and contact information for the trial sponsor	_____1_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____11_____

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
	6b	Explanation for choice of comparators	3
Objectives	7	Specific objectives or hypotheses	4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	___7-8_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	____5_____
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___8_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___8_____

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___8_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	___8_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	___8_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___9_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___9_____

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	__5-7_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___9_____
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Approved HREC application
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9 & clinical trials.gov
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___9_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	____9_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	____10____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	___NA_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___10_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	___10_____
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___10_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	___NA_____
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Approved HREC application
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Approved HREC application
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	5 & Approved HREC application
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	__11_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Approved HREC application
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Approved HREC application
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	__11_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	__NA____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	__NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Approved HREC application

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7 & Approved HREC application

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.