Synopses of clinical trials on non-specific effects of vaccines

A. Randomised controlled trial of early versus late vaccination with BCG to estimate its non-specific effects in infancy

B. 2 x 2 factorial double blind randomised controlled trial to assess the non-specific effects of an additional dose of measles vaccine at 12–16 weeks of age
Protocol Synopsis

<table>
<thead>
<tr>
<th>Item (SPIRIT item no.)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administrative information</td>
<td>Randomized controlled trial of early versus late vaccination with BCG to estimate its non-specific effects in infancy</td>
</tr>
</tbody>
</table>

Introduction

**Description of research question (6a)**

**Hypotheses**

Compared to deferring BCG vaccination until 14 weeks of age, BCG administered within the first 24 hours of birth leads to at least a 15%:

- relative mortality reduction in infants less than 14 weeks of age
- relative risk reduction of severe illness in infants less than 14 weeks of age.

The Bacillus Calmette–Guérin (BCG) vaccine may have additional effects, i.e. it may protect babies from serious infections and death beyond its protection against tuberculosis (TB). However, most of the studies indicating that BCG may have such non-specific effects are observational in nature and their findings are fraught with controversy. This makes it difficult to ascertain whether the babies who get BCG are less prone to severe illness because they receive the vaccine or because they had a lower risk of such illness for other reasons. Moreover, a different set of studies indicates that giving BCG later in infancy, for example at 10 weeks of age, may enhance immune responses against the vaccine and perhaps even to non-mycobacterial antigens. This may enhance any non-specific effects of BCG. The World Health Organization’s Strategic Advisory Group of Experts (WHO SAGE) on Immunization has decided that randomized controlled trials rather than further observational studies are needed to clarify whether BCG has non-specific beneficial effects in increasing infant survival.

Studies in several low- and middle-income countries show that while WHO and most national guidelines state that BCG is to be given as soon as possible after birth, timeliness of BCG vaccination varies widely. Demographic and Health Surveys and Multiple Indicator Cluster Surveys indicate that large proportions of children are vaccinated well after their first week and even after their first month of life. Moreover, BCG vaccination is often deliberately deferred in babies born with low birth weight, many of whom are preterm, and who have a disproportionately high mortality. If, on the one hand, BCG given shortly after birth, including in those born with low birth weight, induces substantial additional

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1 Among children <2 months of age, severe illness (other than TB) will be defined as illness that is associated with any of the following danger signs observed or verified by a study clinician: inability to feed or vomiting of everything, lethargy or unconsciousness, severe lower chest in-drawing, axillary temperature of ≥37.5°C or <35.5°C, grunting, cyanosis, convulsions or a history of convulsions, and/or results in hospitalization and/or results in death.

Among children ≥2 months of age, severe illness (other than TB) will be defined as illness that: is associated with at least one of the following danger signs observed or verified by a study clinician: inability to drink or breastfeed lethargy or unconsciousness, vomiting of all feeds, convulsions or a history of convulsions, and/or results in hospitalization and/or results in death. Hospitalization and death resulting from violent injury or burns will not contribute to the severe illness definition.
survival benefits, a policy change to enforce consistent early vaccination may be required. On the other hand, if deferring BCG vaccination provides even greater survival benefits in the second half of infancy, the scheduling of BCG may need to be reconsidered. Several vaccine candidates against tuberculosis (TB) are under development; two of them are meant to replace BCG and are already in phase II trials. If, in fact, BCG has such non-specific beneficial effects, these new TB vaccines must be evaluated against BCG, also with respect to their effects on child survival.

### Explanation for choice of comparators (6b)

This generic protocol describes a trial that may inform the development of programmatically appropriate scheduling of infant BCG vaccination and providing a benchmark for new TB vaccine candidates by measuring the safety, potential benefits or disadvantages of enforcing babies to be vaccinated within 24 hours of being born in comparison to deferring BCG vaccination to 14 weeks of age.

### Objectives (7)

**Primary objectives:** To compare

1) the risk of death and
2) the risk of severe illness, in the first 14 weeks of life among infants administered BCG at birth (early BCG) with those administered BCG at 14 weeks of age (deferred BCG).

**Secondary objectives:** To compare

1) infant mortality
2) the mortality from 48 hours after randomization until 14 weeks of life
3) the risk of severe illness from 48 hours after randomization until 14 weeks of life
4) production of TNF, IL-1β, IL-6, IL-10, IL-17, IL-22 and IFN-γ in response to mycobacterial (from *Mycobacterium tuberculosis* and PPD) and non-mycobacterial (from *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*) antigens at birth and at 1, 14, 15 and 28 weeks of age among infants who receive early with those who receive deferred BCG. Also other secondary outcomes will be measured (please see below) and can be included in exploratory analyses.

### Trial design

A multi-centre individually randomized placebo-controlled trial

### Methods: Participants, interventions, and outcomes

#### Study setting

A multi-centre study in countries with high mortality/morbidity during early childhood

#### Eligibility criteria

**Inclusion criteria**

A baby born at a participating study clinic or, for sites that also recruit babies born at home, will be included if s/he:

1) has a mother that is of legal age for participation in clinical research studies or is an emancipated minor
2) has a mother/caregiver that resides within the study area, is not intending to move out of the area in the next 4 months and is likely to be traceable for up to 12 months
3) has a mother/caregiver that gives informed consent to trial participation
### Exclusion criteria
A new-born child will be excluded if s/he has:
1. (a) serious congenital malformation(s)
2. severe illness requiring hospitalization
3. a birth weight < 1.8 kg (in African sites) and <1.6 kg (in Asian sites)
4. a mother who is participating or intends to participate in another research study within the next 3 months
5. a mother or other household member with symptoms or signs of TB
6. a severely ill mother with (a) condition(s) requiring hospitalization

### Interventions (11a)

**Experimental group:**
Tubervac® BCG vaccine from the Serum Institute of India or the BCG Vaccine "SSI"® from AJ Biologics, within 24 hours of birth

**Placebo group**
Tubervac® BCG vaccine from the Serum Institute of India or the BCG Vaccine "SSI"® from AJ Biologics, at 14 weeks of age.

Allocation ratio: 1:1

### Outcomes (12)

**Primary outcome measures**
1. Death in the first 14 weeks of life
2. Severe illness in the first 14 weeks of life

**Secondary outcome measures**
1. Death in infancy
2. In a subset: TNF, IL-1β, IL-6 and IFN-γ in response to mycobacterial (M. tuberculosis and PPD) and non-mycobacterial pathogens (E. coli, C. albicans and S. aureus) at 1, 14, 15 and 28 weeks of age
3. Death from 48 h after randomization to 14 weeks of life
4. Severe illness from 48 h after randomization to 14 weeks of life
5. Death in the 14th to the 52nd weeks of life
6. Severe illness in the 14th to the 52nd weeks of life
7. Severe illness in infancy
8. Growth up to 52 weeks of life

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2Consideration should be made to collect specimens (blood cells, DNA, RNA, serum, plasma, urine) from as many babies as ethically and economically possible to enable the subsequent use of these specimens in a nested case-control study. In such a study, babies who die/get severe illness would be cases and a subsample of those who do not would be the controls. In addition to such prespecified epidemiologic analyses, one can use a systems biology approach to explore possible immunobiological mechanisms for any BCG induced NSEs.
Participant timeline (13)

<table>
<thead>
<tr>
<th></th>
<th>Maternity Screening</th>
<th>Postnatal screening (≤24 h of birth)</th>
<th>W 2</th>
<th>W 6</th>
<th>W 10</th>
<th>W 14</th>
<th>W 28</th>
<th>W 52</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother</strong></td>
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<td>Informed consent</td>
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<tr>
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<td>Face to face (clinic) Interview</td>
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<td>X</td>
<td>X</td>
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<td>Telephone interview</td>
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<td>X</td>
<td>X</td>
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<td>Clinical assessment</td>
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<tr>
<td><strong>Maternal Lab tests</strong></td>
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<td>FBC</td>
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<tr>
<td><strong>Infant</strong></td>
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<tr>
<td>Randomization</td>
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<td>Clinical assessment, AE, SAE &amp; anthropometry</td>
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<td><strong>Lab tests on all infants:</strong></td>
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<tr>
<td>CBC</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>X</td>
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<tr>
<td>Stored Blood</td>
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<tr>
<td>Random subsample: Blood cells stimulated with MTB/BCG, PPD, E. coli, S. aureus and C. albicans antigens</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<td>X</td>
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</table>

Sample size (14)

**Hypothesis a)**
The outcome in this hypothesis is death in the first 14 weeks of life. Based on an estimated risk of death ranging from 3% to 1% in the first 14 weeks of life [48], 90% power, 95% confidence and a risk ratio of 0.85, the required sample size per trial arm ranges from 30,003 to 88,468 infants or 60,006 and 176,936 infants in the entire trial. Taking into account a maximum of 10% attrition, the total number of infants required will range from 66,000 to 192,000.

Total trial sample size* (i.e. both trial arms) for 90% power and with 95% confidence; alternative assumptions

<table>
<thead>
<tr>
<th>Risk ratio</th>
<th>0.5%</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
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<tr>
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<td>49,092</td>
<td>32,968</td>
</tr>
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<td>0.85</td>
<td>352,372</td>
<td>176,936</td>
<td>89,230</td>
<td>60,066</td>
</tr>
<tr>
<td>0.90</td>
<td>810,172</td>
<td>402,900</td>
<td>205,324</td>
<td>138,150</td>
</tr>
</tbody>
</table>

*Max 10% for attrition to be added.

**Hypothesis b)**
The outcome in this hypothesis is severe illness in the first 14 weeks of life. Based on an estimated risk of severe illness ranging from 5% to 2% in the first 14 weeks of life, 95% confidence, 90% power and a risk ratio of 0.85, the required sample size per trial arm ranges from 16,025 to 44,615 infants, or from 32,050 to 89,230, infants in the entire trial. Taking into account a maximal of 10% attrition, the total number of infants required will range from 35,500 to 98,500. This shows that the statistical precision of the estimates for protection against severe illness will be
even higher than for death.

Total trial sample size* (both trial arms) for 90% power and with 95% confidence; alternative assumptions

<table>
<thead>
<tr>
<th>Risk ratio</th>
<th>2%</th>
<th>3%</th>
<th>4%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>20,122</td>
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<td>0.8</td>
<td>49,092</td>
<td>32,968</td>
<td>22,502</td>
<td>18,150</td>
</tr>
<tr>
<td>0.85</td>
<td><strong>89,230</strong></td>
<td>60,006</td>
<td>39,712</td>
<td><strong>32,050</strong></td>
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<tr>
<td>0.90</td>
<td>205,324</td>
<td>138,150</td>
<td>104,584</td>
<td>84,460</td>
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</tbody>
</table>

*Max 10% for attrition to be added.

Methods: Intervention assignment

**Allocation sequence generation (16a)**

Eligible infants will be randomized within 24 hours of birth using pre-prepared randomization lists. Randomization is to be stratified by site using permuted blocks of varying size (4, 6 or 8) where eligible infants will be allocated in a 1:1 ratio to one of the two following arms: BCG vaccination within 24 hours of birth (early BCG) or BCG vaccination at week 14 (deferred BCG). All generated randomization lists are to be prepared for each clinic by an independent scientist and kept by an off-site study statistician/epidemiologist. Concealment until the mother-infant pair has been deemed eligible for final inclusion will be ensured using an application running on one dedicated cell phone at each site. It must be ensured that infants within each block receive the same batch of BCG, irrespective of whether they receive early or late BCG.

**Blinding/masking (17a)**

In order to protect randomization, a placebo injection will be given to the babies (in the early vaccination trial arm at 14 weeks of age, in the deferred BCG trial arm at birth). Still, because a high proportion of babies who receive BCG will develop a pustule and scar, it is vitally important that the personnel that record outcomes are rigorously trained and to the extent possible blinded to which trial arm the children belong to. Further, the vaccine should be administered by routine health workers at the health centers that regularly administer the BCG vaccine as well as other childhood vaccines in order to further mask the staff that record the outcomes.

Methods: Data collection, management, and analysis

**Screening of pregnant women and newborns**

Pregnant women between 28 and 40 weeks of gestation residing within the study catchment areas and subsequently their newborns are to be assessed for eligibility.

**Collection of data on baseline features and potential confounders**

Data on relevant baseline characteristics are to be collected during pregnancy and at birth. This will include indicators of socio-demographic characteristics (maternal education and age, food insecurity, income, employment etc.), health (illness history, parity, clinical examination, hemoglobin, BMI, HIV-1 status), postpartum complications, sex of the child, infant feeding practices, and whether the mother received tetanus toxoid during pregnancy as per national recommendations. A careful history deliberately unveiling any TB patients in the infant’s home environment must also be undertaken. It is critically important to precisely capture the participants’ receipt of other vaccines, and encourage the mother to come to well-baby clinics to follow the standard vaccination schedule and carefully keep the booklet in which vaccination dates are captured, bringing it
along during contact with the study staff.

**Follow up and subject retention**

Face to face interviews will be held on the day of birth, and at weeks 14 and 52. Additional interviews will be conducted over the telephone. At each study interview, the outcome assessors shall record whether the baby has died or is alive, and if so, whether (s)he has had severe illness since the last contact. When a study infant has died, the 2014 WHO verbal autopsy questionnaire and narrative interview will be employed to capture the likely cause of infant death.

Home visits shall be made for participants missing clinic visits while those coming to their scheduled clinic visits shall have their transport fees reimbursed. Data on illness, hospital visits and hospitalization shall be collected from interviews with the mother, with more detailed information obtained from hospital records.

**Data collection and management**

All trial procedures must be standardized. Training and refresher sessions are to be scheduled before and during the trial. Pre-coded electronic case report files (CRF)/questionnaires with range and consistency checks will be used. The data capture tool currently used in the ongoing trial on BCG vaccination in HIV-1 exposed babies (Clinicaltrials.gov identifier: NCT02606526) will be made freely available to the WHO from its Ugandan Principal Investigator. The CRFs will be designed, completed, stored and relayed according to Good Clinical Practice (GCP) principles. Entered data shall be checked for consistency and any errors corrected within 24 hours of being entered by study supervisors and, if there are queries, returned to the research assistants for correction within another 24 hours. The outcome data collection team is to be kept separate from the intervention implementation team.

| Statistical methods for analysing primary and secondary outcomes (20a) | Continuous variables with right-skewed distributions should be log-transformed. Means with standard deviations should be used to summarize symmetrically distributed continuous variables, while medians with interquartile ranges should be used for non-normally distributed continuous variables, and percentages for categorical variables. Between-group comparisons for continuous variables that are symmetrically distributed should be made using t-tests or, if adjustment for other variables are required, linear regression, while the Wilcoxon rank sum test should be used to compare continuous variables where even their log-transformed values remains non-symmetrically distributed. Group comparisons for proportions should use chi-square tests. Two-sided statistical tests and 95% confidence intervals for descriptive results, effect estimates and safety estimates should be used. All relevant data; from both scheduled and unscheduled visits should be included in the analysis.

Primary analysis: All randomized children are to be included in the primary, i.e. intention-to-treat, analysis. The main study outcomes are death and severe illness (of which death is one defining outcome) during the first 14 weeks of life. Although the sample size estimations are based on specified relative risks and these should be estimated, children that are lost to follow-up should be included in survival analyses, censoring them during periods when data could not be recorded. Survival analyses shall include Kaplan-Meier plots and log-rank tests for descriptive statistics while Cox proportional hazards regression models shall be used to estimate the adjusted effect of the intervention on child death.

For the severe illness outcome, the final analysis should also use incidence density and Poisson regression or, in case of over-dispersion, negative bimomial
regression analyses, to estimate incidence rate ratios (IRR), enabling the capture of more than one illness event per child. The effect in percent is 100×(1-RR), 100×(1-IRR) or 100×(1-HR).

For the immunological readouts, if transformation can yield reasonably symmetrical distributions, T-tests or linear regression should be used to compare responses between the trial limbs. Adjustment for potential confounders should be done in the unlikely event that there is a combination of: 1) baseline imbalances between trial limbs, and 2) the variables for which there are such baseline differences are strongly associated with the study event.

It is likely that some of the babies randomized to receive deferred BCG may still receive BCG before their scheduled 14 week dose. In addition to the intention-to-treat analysis where outcomes should be compared according to the random allocation, instrumental variable analyses can be conducted in an attempt to even better estimate biological/causal effects of the actual receipt of the vaccine. In these analyses, random allocation should be the instrument. To enable such analyses, actual receipt as well as the age of BCG vaccination should be captured in all participants. Also per protocol and as treated analyses can be considered.

Statistical methods for any additional analyses (20b)

<table>
<thead>
<tr>
<th></th>
<th>1) As a hypothesis generating effort to understand the latency period after which BCG may induce non-specific effects, estimate the protection against severe illness during the first 14 weeks of life on a sliding scale starting from randomization until 7 completed days post-randomization.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2) Effect of receiving early versus deferred BCG on the secondary outcomes not specifically mentioned elsewhere in this protocol.</td>
</tr>
<tr>
<td></td>
<td>3) Safety will be analysed according to type, frequency and severity of adverse events (AEs) that occur in children during the trial.</td>
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<td></td>
<td>4) Sub-group analysis for potential effect measure modification will be on the strata defined by low (&lt;2,500 g) or normal (≥2,500 g) birth weight, the babies’ sex and whether the mother had HIV-1 infection when the baby was born or not, in addition to other baseline characteristics which may interact with early versus deferred BCG administration. Such subgroup/interaction analyses must be defined based on available literature and considerations before embarking on the analyses; it is not to be driven by study data. They must be described in an analysis plan which should be made publically available before data analysis.</td>
</tr>
</tbody>
</table>

Methods: Monitoring

Data monitoring (21a) **Data Monitoring Committee (DMC):** A group of independent scientists with expert knowledge and experience in paediatrics, immunology, TB and statistics shall form a DMC. After meeting shortly before trial start, it shall periodically and at least annually, review and assess available study data for safety, conduct and efficacy. The DMC shall advise the project management on study continuation, modification or termination based on its reviews and pre-established stopping rules. The DMC is to review the data yearly for safety and interim analysis for the main outcomes taking into account the DAMOCLES group recommendations should be performed when approximately half of all the expected deaths have been recorded, or when requested by the DMC.

**Auditing:** The study shall be audited by an independent auditor or monitored by (a) participating scientist(s) not involved in the day-to-day running of the trial before study start (site-readiness), and thereafter annually, as appropriate for each site.
**Protocol amendments:** Important protocol modifications (such as those resulting from changes to eligibility criteria, outcomes, and planned analyses) shall be communicated to and discussed among the investigators, discussed with the DMC and, when appropriate, the ethics committees. Such modifications shall also be reflected in amendments to the description in the trials registry.

**Adverse events (22)**

**AE reporting/ Clinical and safety monitoring**

An AE is any harmful manifestation occurring in a trial participant, whether this manifestation is believed to be related to the study BCG vaccine or not. AEs include localized abscess formation at the injection site, suppurative lymphadenitis and disseminated BCG infection.

**AE monitoring, recording and reporting**

Potential AEs shall be carefully monitored throughout the trial with specific questionnaires. The mothers shall be invited and encouraged to consult the study clinic in case of any disease or symptoms that arise between visits. AEs shall be investigated at each follow-up visit and can be reported spontaneously or in response to general, non-directed discussions with the attending midwife/researcher or physician/researcher. All AEs, regardless of seriousness, severity, or presumed relationship to study therapy, shall be recorded using medical terminology in the source document and on the AE form. Whenever possible, diagnoses shall be given when signs and symptoms are due to a common aetiology. Investigators shall record their opinion concerning the relationship of the AE to BCG vaccination on the AE form. All AEs shall be followed up until resolution or until a stable clinical end-point is reached. All measures required for AE management and the ultimate outcome of the AE shall be recorded in the source document and reported on the AE page.

**Serious Adverse Event reporting**

When the investigator, or trained doctor, becomes aware that a serious AE (SAE) has occurred, the appropriate reporting form shall be completed, and a copy emailed to the DMC and the local Institutional Review Board within 48 hours.

**Management of SAE**

In case of illness, the mother shall be encouraged to immediately contact the research unit and/or to bring the infant immediately to the research unit. Infants shall be seen by one of the research midwives/ paediatricians and appropriate medical or surgical interventions shall be provided. Insurance coverage shall be made by a pre-specified Insurance company, and if any participant is harmed as a result of the BCG vaccine (within 2 years of the vaccination) she/he shall be appropriately compensated.

**Ethics and dissemination (omitted)**

**Appendices**

**Informed consent (32)**

Written individual informed consent in vernacular is to be obtained from each of the participating mothers by trained study staff. The consent process will explain the nature of the study, the risks and benefits of participating, the intervention and that intervention allocation is by a random process. In situations requiring translation or in cases where the mother is unable to read and write, the consent process will take place in the presence of an independent third person, who will act as a witness and also co-sign the consent form. Additional consent will be obtained from study participants for the collection and storage of blood specimens for ancillary studies. Confidentiality of information and the right of the participant to withdraw from the study at any time during the study will be explained to the mothers. All study staff will be trained on participant
confidentiality and autonomy.

Ethics permission to conduct the study is to be obtained from all relevant local and international ethical committees/bodies.

| Biological specimens (33)   | • Entire study sample: Blood (Sera, +/- PBMC) at: birth, weeks 14 & 52  
|                           | • Sub-sample: Blood (Sera, +/- PBMC) at: birth, and weeks 2, 14, 15, 28 & 52 |
Protocol Synopsis

<table>
<thead>
<tr>
<th>Item (SPIRIT item no.)</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td><strong>Administrative information</strong></td>
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<tr>
<td>Title (1)</td>
<td>2 x 2 factorial double blind randomised controlled trial to assess the heterologous effects of an additional dose of measles vaccine at 12-16 weeks of age</td>
</tr>
</tbody>
</table>

**Introduction**

Description of research question (6a)  

_Hypotheses:_

1. Receipt of an earlier additional dose of measles vaccine at 14 weeks of age reduces the risk of subsequent death.

2. There is no difference in all-cause mortality between a schedule with penta-valent vaccine (DTwP-HBV/Hib) at 10 weeks and a schedule with DTwP-HBV/Hib at 9 months

_Existing knowledge:_

Over the last century vaccines have proven to be one of the greatest benefactors to public health with an estimated 2-3 million deaths world-wide directly prevented each year by vaccination. Interestingly, there is a growing body of evidence in humans to show that certain vaccines may have effects that are unrelated to those directly targeted by the vaccine. These effects are often referred to as heterologous or non-specific effects. It has been hypothesised that live vaccines, such as the measles vaccine, have beneficial heterologous effects.

Measles vaccines have been in use for over 50 years, with a corresponding reduction in measles related child illness and death observed globally. There are some studies however, to suggest that measles vaccination has reduced child mortality by a greater magnitude than would be expected by the prevention of measles disease alone. A recent systematic review has analysed the current evidence for this effect on all-cause mortality, although most of the data included were considered to be of poor quality, with few randomised controlled trials existing, and a lack of geographical diversity. Within these limitations, study authors reported that standard-titre measles vaccines may reduce risk of all-cause mortality by as much as 50%.

The immunological evidence in humans for heterologous effects of measles vaccines is limited. And although a systematic review on immunological heterologous effects of childhood vaccination identified 14 studies where a measles vaccine was used, these studies were very heterogeneous in design and in the outcomes that were measured. Of note one study did perform measures which appeared clinically relevant, showing an increase in immune cell proliferation to tetanus toxoid and _Candida spp. in vitro_ following measles vaccination. How this relates however, to prevention of non-measles diseases in the clinical context is not known. More convincing
Evidence for the heterologous effects of measles vaccine is actually provided by a study which examines the indirect consequences of measles infection. This study shows that historically in the UK, Denmark, and United States there has been a positive correlation between the incidence of measles infections and non-measles infections. A finding which is supported by immunological studies in non-human primates which show measles infection results in a prolonged state of immune suppression. That is, measles infection leads to an immune suppressed state that makes one more susceptible to subsequent non-measles infections.

The current framework of the expanded programme on immunisation (EPI) is set around the delivery of DTP, pneumococcal conjugate, *Haemophilus influenzae* type B, and hepatitis B vaccines as three priming doses early in infancy. For some of these vaccines there is a growing body of evidence however, that delivery as two priming doses in early infancy followed by a boosting dose (2p+1 schedule) in late infancy generates greater persistence of antibody responses. Such a schedule is likely to bring significant cost savings to vaccine delivery as one vaccine visit will be removed.

**Need for a trial:**

Therefore, there is some epidemiological evidence for heterologous effects of measles vaccination which may be due to immuno-stimulatory actions of the vaccine and/or in part due to the prevention of the measles induced immune suppressive effect and susceptibility to non-measles infections. However, the evidence is not strong enough to inform any changes to vaccination policy at either a local or international level. There is also an impending shift towards a two-priming followed by booster dose EPI frame-work where there is likely to be clear tangible benefits in immunogenicity and costs, however the impact of such a schedule on heterologous effects is an area of uncertainty. As such studies which aim to assess the heterologous effects of measles vaccines in the context of a 2p+1 EPI are needed.

<table>
<thead>
<tr>
<th>Explanation for choice of comparators (6b)</th>
<th>Primary:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The comparator arm is the current EPI infant vaccine schedule which includes the first dose of DTwP-HBV/Hib at 6 weeks of age and has subsequent visits at a minimum 4-week interval (3+0 schedule).</td>
<td>1. To determine the effect on mortality &lt;2 years of an infant schedule with an additional early dose of measles vaccine given with DTwP-HBV/Hib at 14 weeks of age; arms B+D vs A+C</td>
</tr>
<tr>
<td></td>
<td>2. To determine the impact on mortality &lt;2 years of moving DTwP-HBV/Hib at 10 weeks to DTwP-HBV/Hib at 9 months; arms A+C vs arms B+D</td>
</tr>
</tbody>
</table>
Secondary:
1. To determine the impact of 2+1 schedule vs 3+0 on pertussis disease (arms A+B vs. C+D)
2. To determine the impact of early MV at 14 weeks on measles
3. To determine the effect of the primary research objectives on cause-specific mortality
4. To determine the effect of the primary research objectives on morbidity outcomes such as all cause-hospitalization or hospitalization for acute febrile illness
5. To determine whether there is a sex-differential effect for the primary research questions
6. To determine the additional programmatic and financial implications

Immunological research questions (nested study):
1. To determine the serum IgG geometric mean concentrations against DTwP-HBV/Hib antigens one month following the second priming dose, at 9 months of age, one month following the 9-month booster, and at 2 years of age for each of the study groups.
2. To determine which immunological measures correlate with morbidity outcomes.

Trial design (8)
Individually randomised, multi-centre, international, placebo-controlled, 2 x 2 factorial clinical trial, with nested immunological sub-study.

See Table 1 for description of study arms

Methods: Participants, interventions, and outcomes

<table>
<thead>
<tr>
<th>Study setting</th>
<th>The study will be conducted in African and Asian settings of high mortality/morbidity (greater than 1.5% mortality between 14 weeks and 2 years of age) with an infant immunisation programme in place in line with WHO recommendations.</th>
</tr>
</thead>
</table>
| Eligibility criteria | Participants will be children who are eligible for their 10-week vaccination visit (DTP2) who meet the following inclusion and exclusion criteria.  

Inclusion criteria:  
Participants meeting all of the following criteria will be enrolled in the study
- aged less than 14 weeks  
- in good health as judged by medical history and physical exam  
- has received birth dose and DTP1 vaccinations as per EPI schedule  
- parent or caregiver willing and able to give informed consent |
**Exclusion criteria:**  
*Participants meeting any of the following criteria will not be enrolled in the study*

- fever ≥ 38 degrees
- requiring immediate hospitalisation
- known allergy to any vaccine
- receipt of other investigational vaccine prior to enrolment
- participant’s family plans to move outside study area
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participant at risk because of participation in the trial, or may influence the result of the trial, or the participant’s ability to participate in the trial.

**Temporary exclusion criteria/delayed immunisation**

- In the event of systemic illness or fever ≥ 38 degrees Celsius at the time of any visit then immunisation will be deferred and rearranged as appropriate when the participant is recovered.
- If in the opinion of the investigator the illness is judged to require further assessment or treatment, then the participant will be referred to the appropriate medical staff. Minor illness (e.g. upper respiratory illness without fever above 38 degrees Celsius) is not a reason to temporarily exclude from participation.

<table>
<thead>
<tr>
<th>Interventions (11a)</th>
<th>Experimental group:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants will be randomised to receive either their EPI vaccination in a 2p+1 or 3p+0 schedule, and, additionally, either a measles or placebo vaccine at approximately 14 weeks of age</td>
<td></td>
</tr>
<tr>
<td>See Table 1 for description of study arms</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes (12)</th>
<th>Primary outcome:</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality.</td>
<td></td>
</tr>
<tr>
<td>For the comparison of the effect of the additional measles dose, this will be defined as: death occurring between the third dose of DTP containing vaccine (DTP3) (~14 weeks of age) and 2 years of age. Non-disease related deaths for example trauma and burns deaths will be excluded.</td>
<td></td>
</tr>
<tr>
<td>For the comparison of the 2p+1 vs 3p+0 schedules this will be defined as: death between randomisation (14 weeks) and 2 years of age. Non-disease related deaths for example trauma and burns deaths will be excluded.</td>
<td></td>
</tr>
</tbody>
</table>
Secondary outcomes:
- pertussis
- measles
- cause-specific mortality
- all-cause morbidity defined as health seeking behaviour such as hospitalisation, consultation with health clinic, or other health professional; consideration of other endpoints such as hospitalization for acute febrile illness
- all-cause and cause-specific hospitalisations
- mortality and morbidity up until 5 years of age in a subset of children

Immunological sub-study:
- immunogenicity to vaccine antigens
- immune cell phenotyping of a subset of participants in each arm correlated with non-specific outcomes
- Genome, epigenome, and transcriptome of a subset of participants in each arm correlated with non-specific outcomes

Participant timeline (13)
Participants will be randomised upon recruitment at 10 weeks of age and be followed up until their 2nd birthday. Visits will occur in line with the vaccination schedule at age 14 weeks, 9 months and 12 months.

Sample size (14)
Sample size calculations:

For mortality from 14 weeks to 2 years of age; a sample size of 21,836 per arm (total study size 87,344) will be sufficient to detect a minimum relative difference of RR=0.8 with 90% power and two-sided alpha of 0.025, assuming mortality in the EPI arm of 2.5%. This sample size is sufficient to detect an interaction between vaccine group and sex of at least OR: 1.7 (See Table 2).

Immunogenicity sub study: Samples from 250 participants per arm (1000 samples in total) will provide 90% power to detect a 35% increase in antibody following the 9-month booster. Sub-studies will be conducted in Africa and Asia separately.

Methods: Intervention assignment
Allocation sequence generation (16a)
Block randomisation with randomly varying block size, stratified by sex and geographic location.

Blinding/masking (17a)
Placebo: saline (IM or SC)

Methods: Data collection, management, and analysis
Data assessment and collection (18a)
- Accurate recording of admission and discharge diagnoses (including death) at hospitals
- Study visits will occur at age 10 weeks, 14 weeks, and 9 and 12 months to coincide with vaccination visits. Additional visits to collect blood samples will occur for a subset at week 18 and month 10.
• telephone follow up will occur every three months to:
  o confirm participant is still happy to continue with the study
  o ensure participant and family still lives in the area
  o screen for serious adverse events/mortality
  o encourage attendance at study clinic/hospital in the event of major illness

  • Blood samples to be taken in clinic in a subset of participants at: 18 weeks, 9, 10, 12, and 24 months

<table>
<thead>
<tr>
<th>Statistical methods for analysing primary and secondary outcomes (20a)</th>
<th>For mortality outcomes proportional hazards models will be used adjusted for sex, randomised group and include a group by sex interaction term. Participants who are lost to follow up will be censored in the analysis at the time of last known contact. For the analysis of morbidity outcomes, Poisson or negative binomial regression models will be used, depending on data over-dispersion. For each participant, the number of events during the follow up period will be the outcome of interest. Models will adjust for geographic region, sex, randomised group, and include a group by sex interaction. The length of follow up time available for each participant will be included as an offset in the model.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical methods for any additional analyses (20b)</td>
<td><strong>Immunology sub-study:</strong> Banked blood samples will be analysed to determine which immunological measures correlate with differences in the epidemiological outcomes.</td>
</tr>
<tr>
<td>Methods: Monitoring</td>
<td>A multidisciplinary DSMB will be convened which will meet at least yearly to monitor interim safety and outcome data, and review assumptions underlying sample size calculations.</td>
</tr>
<tr>
<td>Adverse events (22)</td>
<td>Health status of participants will be monitored and reported as study outcomes.</td>
</tr>
<tr>
<td>Ethics and dissemination (omitted)</td>
<td></td>
</tr>
<tr>
<td>Appendices</td>
<td></td>
</tr>
<tr>
<td>Informed consent (32)</td>
<td>Written informed consent – signed or fingerprint</td>
</tr>
<tr>
<td>Biological specimens (33)</td>
<td>Blood (Sera, +/- PBMC, DNA, and RNA) at: 18 weeks, 9, 10, 12, and 24 months</td>
</tr>
</tbody>
</table>
Appendix

Table 1: Schedule of administration

<table>
<thead>
<tr>
<th>ARM A (current EPI)</th>
<th>Schedule</th>
<th>W 10</th>
<th>W 14</th>
<th>M 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>3p+0 schedule</td>
<td>D</td>
<td>D</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>3p+0 schedule</td>
<td>D</td>
<td>D</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>2p+1 schedule</td>
<td></td>
<td>D</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>2p+1 schedule</td>
<td></td>
<td>D</td>
<td>M</td>
<td></td>
</tr>
</tbody>
</table>

Standard EPI vaccination schedule containing: DTwP-HBV/Hib: Diphtheria, tetanus, pertussis (whole cell formulation), hepatitis B, and *Haemophilus influenzae* type b; and pneumococcal conjugate vaccine.

Placebo version of DTwP-HBV/Hib

Measles and rubella vaccine

Placebo version of measles and rubella vaccine

Table 2: Sample size calculations

<table>
<thead>
<tr>
<th>Assumptions</th>
<th>Mortality* in EPI arm (ARM A)</th>
<th>Diff % (RR 0.8)</th>
<th>N per arm</th>
<th>Total study size – 4 arms</th>
<th>OR for sex diff detectable with this sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha = 0.025 (two-sided), 90% power</td>
<td>1.5%</td>
<td>0.3%</td>
<td>36,729</td>
<td>146,916</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>2.0%</td>
<td>0.4%</td>
<td>27,421</td>
<td>109,684</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>0.5%</td>
<td>21,836</td>
<td>87,344</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* from 14 weeks to 2 years of age