Impact of BCG revaccination on the response to unrelated vaccines in a Ugandan adolescent birth cohort: randomised controlled trial protocol C for the ‘POPulation differences in VACcine responses’ (POPVAC) programme

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ABSTRACT

Introduction There is evidence that BCG immunisation may protect against unrelated infectious illnesses. This has led to the postulation that administering BCG before unrelated vaccines may enhance responses to these vaccines. This might also model effects of BCG on unrelated infections.

Methods and analysis To test this hypothesis, we have designed a randomised controlled trial of BCG versus no BCG immunisation to determine the effect of BCG on subsequent unrelated vaccines, among 300 adolescents (aged 13–17 years) from a Ugandan birth cohort. Our schedule will comprise three main immunisation days (week 0, week 4 and week 28): BCG (or no BCG) revaccination at week 0; yellow fever (YF-17D), oral typhoid (Ty21a) and human papillomavirus (HPV) prime at week 4; and HPV boost and tetanus/diphtheria (Td) boost at week 28. Primary outcomes are anti-YF-17D neutralising antibody titres, Salmonella typhi lipopolysaccharide-specific IgG concentration, IgG specific for L1-proteins of HPV-16/HPV-18 and tetanus and diphtheria toxoid-specific IgG concentration, all assessed at 4 weeks after immunisation with YF, Ty21a, HPV and Td, respectively. Secondary analyses will determine effects on correlates of protective immunity (where recognised correlates exist), on vaccine response waning and on whether there are differential effects on priming versus boosting immunisations. We will also conduct exploratory immunology assays among subsets of participants to further characterise effects of BCG revaccination on vaccine responses. Further analyses will assess which life course exposures influence vaccine responses in adolescence.

Strengths and limitations of this study

- This will be the first well-powered trial to investigate effects of BCG revaccination on responses to unrelated vaccines in adolescents.
- Effects on both live-attenuated and inert vaccines will be studied.
- Our robust immunoepidemiological design and nested immunological studies will address specific hypotheses regarding pathways of effects of BCG immunisation on unrelated vaccine responses.
- One limitation is that interaction between the three vaccines administered together 1 month after BCG immunisation may mask the true effect of BCG revaccination on individual vaccine responses.

Ethics and dissemination Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be shared with Uganda Ministry of Health, relevant district councils, community leaders and study participants. Further dissemination will be done through conference proceedings and publications.

Trial registration number ISRCTN10482904.

INTRODUCTION

There is increasing evidence that BCG immunisation has non-specific, protective effects relating to infections other than tuberculosis. Experimental studies using BCG suggest that effects on the innate immune...
response are an important component of this phenomenon: BCG immunisation induces lasting epigenetic modification of innate immune cells, including monocytes, macrophages and natural killer cells. This process, by which the innate immune system develops a form of memory, has been called ‘trained innate immunity’. Evidence that a range of stimuli, including bacterial products (particularly Salmonella typhi lipopolysaccharide (LPS)), and infections, including malaria and hepatitis B, may induce trained innate immunity; that the profile into which cells are trained varies with the dose and characteristics of the stimulus; and that effects may be induced prenatally (on exposure to maternal infections) as well as later in life is accumulating.

It is plausible that variation in the intensity and spectrum of experience of previous infections, and hence the epigenetic programming and consequent functional profiles of innate immune cells, contributes to the many differences in immunological activity observed between geographically and environmentally distinct settings, and hence to differences in vaccine response. If this hypothesis is correct, BCG immunisation can act as a model for the effects of prior infection and may also be a tool for inducing enhanced benefits for other vaccines. Vaccine-specific responses can also act as a model for responses to infection. This is especially relevant given the current interest in the potential benefit of BCG immunisation against COVID-19 disease.

In Europe, BCG vaccination 2 weeks before influenza vaccination has been shown to result in enhanced antibody responses to influenza proteins. BCG immunisation 4 weeks before yellow fever (YF 17D) vaccination has also been found to result in reduced replication of the YF vaccine virus; this was not associated with a significant reduction in the desired neutralising antibody response to YF or in the interferon-γ response, but the study size was small and may not have had sufficient power to demonstrate important effects.

In Uganda, BCG immunisation at birth is recommended. The benefits of BCG immunisation in adolescence for protection against tuberculosis are not known and may differ between settings. Whether BCG immunisation in adolescents in Uganda will have non-specific effects on the innate immune response, on subsequent immunisations and (indeed) on general health (given the prior exposure at birth and the ongoing exposure to non-tuberculous mycobacteria and other infections) is not known. In protocol C of the ‘POPulation differences in VACcine responses’ programme (POPVAC C), we plan to address this knowledge gap by randomising adolescent members of the Entebbe Mother and Baby Study (EMaBS) birth cohort in a nested trial of BCG revaccination versus no BCG revaccination before immunisation with other vaccines. We summarise the protocol here.

**HYPOTHESIS**

The overarching goal of the POPVAC programme is to understand population differences in vaccine responses in Uganda, in order to identify strategies through which vaccine effectiveness can be optimised for the low-income, tropical settings where they are especially needed. This trial C is one of three parallel trials whose designs and cross-cutting analyses are described separately in this journal (bmjopen-2020-040425, bmjopen-2020-040426 and bmjopen-2020-040427). For this trial C, we address the concept of trained innate immunity through the hypothesis that BCG immunisation modifies the response to subsequent unrelated vaccines.

**OBJECTIVE**

To determine whether BCG revaccination modulates the response to unrelated vaccines among Ugandan adolescents.

**METHODS AND ANALYSIS**

**Setting and participants**

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting guidelines are used. This trial will be a randomised, controlled, open, parallel group trial investigating the effect of BCG revaccination on unrelated vaccine response outcomes. The study will take place in Entebbe municipality, Wakiso district, Uganda, and will involve participants in the EMaBS birth cohort. In EMaBS, a cohort of 2500 pregnant women were recruited between 2003 and 2005 for a trial of anthelmintic treatment during pregnancy and early childhood, investigating effects on childhood vaccine responses and infectious disease incidence. We aim to enrol 300 EMaBS birth cohort participants, randomising 150 to each intervention arm. All EMaBS participants received BCG at birth; hence, the current trial participants (in the BCG intervention arm) will undergo revaccination. EMaBS participants are expected to be aged 13–17 during recruitment to this study. As part of the ongoing cohort follow-up, participants will be encouraged to attend the clinic for interim illness events, and all serious adverse events, including hospitalisations, will be documented.

**Recruitment criteria**

**Inclusion criteria**

1. A participant of the EMaBS.
2. Written informed consent by parent or guardian.
3. Written informed assent by participant.
4. Willing to remain in the study area for the duration of the study.
5. Willing to provide locator information and to be contacted during the course of the trial.
6. Women agree to avoid pregnancy for the duration of the trial.
7. Able and willing (in the investigator’s opinion) to comply with all the study requirements.

Exclusion criteria

1. Concurrent enrolment into another clinical trial.
2. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and neurological illness.
3. A history of serious psychiatric condition or disorder.
4. Moderate or severe acute illness characterised by any of the following symptoms: fever, impaired consciousness, convulsions, difficulty in breathing and vomiting, or as determined by the attending project clinician.
5. A history of previous immunisation with YF, oral typhoid (Ty21a) or human papillomavirus (HPV) vaccine; previous immunisation with BCG or tetanus/diphtheria (Td) vaccine at age ≥ 25 years.
6. Concurrent oral or systemic steroid medication or the concurrent use of other immunosuppressive agents within 2 months prior to enrolment.
7. A history of allergic reaction to immunisation or any allergy likely to be exacerbated by any component of the study vaccines, including egg or chicken proteins.
8. Tendency to develop keloid scars.
9. Positive HIV serology.
10. Positive pregnancy test.
11. Women currently lactating, with confirmed pregnancy or with intention to become pregnant during the trial period.
12. Use of an investigational medicinal product or non-registered drug, live vaccine or medical device other than the study vaccines for 30 days prior to dosing with the study vaccine, or planned use during the study period.
13. Administration of immunoglobulins and/or any blood products within the 3 months preceding the planned trial immunisation date.

Interventions

We will randomise participants to receive BCG or not to receive BCG 4 weeks before immunisation with a panel of licensed unrelated vaccines (discussed below). The adolescents in the intervention arm will receive a dose of 0.1 mL of BCG-Russia (Serum Institute of India) in the deltoid region of the right upper arm.

Randomisation and allocation to treatment arm

An independent statistician will generate the randomisation code using a randomly permuted block size. This code will be embedded as a web-based randomisation system in REDCap (Research Electronic Data Capture) software. Randomisation to the two trial arms will be done in a 1:1 ratio. At enrolment, eligibility criteria will be checked and eligible participants will be allocated sequentially to the next randomisation number, with the corresponding trial arm designated in REDCap. The randomisation code will be kept securely by the trial statistician with a second copy held by a data manager or statistician not otherwise involved in the trial at the MRC/UVRI and LSHTM Uganda Research Unit.

Blinding

This trial will not be blinded to clinicians or participants because they will not participate in outcome ascertainment, and the expected development of a BCG skin reaction makes blinding difficult. It is unlikely that participants allocated to ‘no BCG’ will seek this privately. Only laboratory personnel evaluating vaccine response outcomes will be unaware of BCG allocation, so outcome ascertainment will not be biased through lack of blinding.

Immunisations

We anticipate that BCG revaccination may have different effects on live and non-live, oral and parenteral, and priming and boosting vaccines. Activated innate responses may kill live vaccines and suppress subsequent adaptive responses by this or other mechanisms, but bias, or even enhance, responses to toxoids or proteins; thus, results from a single-vaccine study would not be generalisable.

We therefore propose to study a portfolio of licensed vaccines (live and inert, oral and parental, priming and boosting) expected to be beneficial (in some cases, already given) to adolescents in Uganda. Our schedule table 1 and online supplemental table S1 will comprise three main immunisation days (week 0, week 4 and week 28). Additional HPV immunisation will be provided for girls aged ≥14 years, and a second Td boost will be given after completion of the study to accord with the national Expanded Programme on Immunisation (EPI) routines, but the response to these will not specifically be addressed. Further rationale for the selection of vaccines is detailed in online supplemental information. Our schedule has been developed in consultation with the EPI programme and is cognisant of potential interference between vaccines.

Schedule of immunisation and sampling

The schedule of immunisation and sampling is outlined in online supplemental table S1. While optimal timings for outcome measures vary between vaccines, sampling at 8 weeks after BCG and at 4 weeks after YF-17D, Ty21a, HPV and Td is proposed for the primary end points, targeting the establishment of memory responses and approximate peak of antibody responses. A secondary end point at 1 year will assess waning. All analyses will take baseline measurements into account. Immunisation postponement criteria are detailed in online supplemental information.

Outcomes

Primary outcomes

These will be assessed in all participants.
1. YF-17D: Neutralising antibody titres (plaque-reduction neutralisation test) at 4 weeks after YF immunisation. Ziriminya L, et al. BMJ Open 2021;11:e040430. doi:10.1136/bmjopen-2020-040430
2. Ty21a: *Salmonella typhi* LPS-specific IgG concentration at 4 weeks after Ty21a immunisation.
3. HPV: IgG specific for L1-proteins of HPV-16/HPV-18 at 4 weeks after HPV priming immunisation.
4. Td: Tetanus and diphtheria toxoid-specific IgG concentration at 4 weeks after Td immunisation.

Secondary outcomes
These will be assessed in all participants and will further investigate estimates of protective immunity (for vaccines where these are available) and dynamics of the vaccine responses, as well as the impact of the interventions on parasite clearance.

1. Protective immunity: Proportions with protective neutralising antibody (YF), protective IgG levels (TT) and seroconversion rates (Ty21a) at 4 weeks after the corresponding immunisation.
2. Response waning: Primary outcome measures (all vaccines) repeated at week 52 and area-under-the-curve analyses. Parasitic infection may accelerate, and anti-parasitic interventions may delay, waning.
3. Priming versus boosting: Effects on priming versus boosting will be examined for HPV only, comparing outcomes 4 weeks after the first and 4 weeks after the second vaccine dose.

Furthermore, our sample collection will offer opportunities for an array of exploratory immunological evaluations on stored samples, focusing mainly on vaccine antigen-specific outcomes. Exploratory assays will provide further detail on the mechanisms underlying effects of BCG on responses to unrelated vaccines. Such assays will assess the effects of revaccination with BCG on the profile of cellular phenotypes established before immunisation with the later-scheduled vaccines. For example, samples collected will provide opportunities for profiling using mass and flow cytometry, markers of immune activation and regulation, and gene expression studies.

Additional measurements
Other additional assays are discussed in online supplemental information and will comprise evaluation of helminth and malaria infection exposure, HIV serology (at baseline), pregnancy and full blood count testing (at baseline and before immunisation on each immunisation day).

Sample size considerations
Based on the literature and preliminary data, we anticipate that SDs of primary outcome measures will lie between 0.3 and 0.6 log$_{10}$ and that revaccination with BCG may increase responses by approximately 0.12–0.14 log$_{10}$. Based on these assumptions, we aim to enrol 300 EMaBS participants (150 BCG revaccination, 150 non-BCG revaccination). Allowing for 10% loss to follow-up, this will give over 90% power to detect a difference of 0.12 log$_{10}$ in vaccine response between the pre-BCG immunised and non-pre-BCG immunised groups at 5% significance level and assuming vaccine response SD of 0.3 log$_{10}$ (table 2).

Ethics and dissemination
Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus Research Institute (reference: GC/127/19/05/682), the London School of Hygiene and Tropical Medicine (reference: 16034), the Uganda National Council for Science and Technology (reference: HS 2491) and from the Uganda National Drug Authority (certificate number: CTA00094). Any protocol amendments will be submitted to ethics committees and regulatory bodies for approval before implementation.

Participants will be adolescents and therefore a vulnerable human population. Care will be taken to provide adequate age-appropriate and education-status-appropriate information, to ensure that it is understood and to emphasise that participation is voluntary. Participants will be enrolled only when they have given their own assent and when consent has been given by the parent or guardian. No major risks to the participants are anticipated as all the vaccines to be given are licensed and known to be safe.

Regarding BCG immunisation or revaccination in adolescence, benefits with respect to protection against...
tuberculosis among Ugandan adolescents are unknown and may, at best, be modest. There may be non-specific benefits. WHO’s SAGE committee concluded, in their summary of October 2017, that

BCG revaccination is safe in *Mycobacterium tuberculosis* infected and uninfected populations. There is a lack of evidence from randomised controlled trials and retrospective cohort and case-control studies demonstrating the efficacy and effectiveness of BCG revaccination in adolescents and adults after primary BCG vaccination in infancy for protection against TB disease. Due to absence of evidence, BCG revaccination is not considered cost-effective. Further research is warranted to explore whether certain sub-groups of age, geographic or *M. tuberculosis* exposure categories would benefit from BCG revaccination.

We hope, through this work, to contribute to this debate.

Study findings will be published through open access peer-reviewed journals and presentations at local, national and international conferences and to the local community through community meetings. Anonymised participant-level data sets generated will be available on request.

**Patient and public involvement**

The EMaBS research team has previously worked with volunteer local council field workers to ensure regular follow-up of participants, and these field workers continue to attend participants’ meetings and provide a mechanism by which the communities from which participants are drawn can be informed about ongoing work. In addition, prior to the start of this study, we will share our plans with district health and education officers and with colleagues at Entebbe Hospital. We will establish an advisory committee of parents who will help us ensure that EMaBS cohort members can participate in the study without undue disruption to their school work. Study findings will be shared with these stakeholders and with participants.

**Data management and analysis**

Sociodemographic information and clinical and laboratory measurements will be recorded and managed using REDCap tools, with paper-based forms as backup. All data will be recorded under a unique study ID number. When paper forms must be used, data will be double-entered in a study-specific database, with standard checks for discrepancies. All data for analysis will be anonymised and stored on a secure and password-protected server, with access limited to essential research personnel.

The effect of BCG versus no BCG revaccination on the outcomes will be analysed, including subgroup analysis by sex. The analysis will test whether BCG preimmunisation alters the response to live or inert vaccines given after 4 weeks, including effects on vaccine replication, immune response profile, priming, boosting and waning. It will indicate whether including BCG as a component of school-based immunisation schedules is likely to have non-specific benefits for Ugandan adolescents.

**DISCUSSION**

It is increasingly clear that several live vaccines, including BCG, measles vaccine and Vaccinia (smallpox) vaccine, have non-specific, beneficial, effects, including reduced mortality (not related to the infectious disease that they were designed to target). The potential effects of BCG on responses to unrelated vaccines, specifically on live-attenuated ones such as YF and Ty21a, might model its effects on responses to unrelated infectious agents. In contrast, non-specific negative effects have been associated with inactivated vaccines such as diphtheria–tetanus–pertussis (DTP). A high childhood mortality has been observed among girls vaccinated with DTP. It has been further suggested that reducing time of exposure to DTP as the most recent vaccination with BCG may reduce this childhood mortality.

We hypothesise that BCG immunisation both achieves non-specific benefits and influences vaccine responses through mechanisms based on effects on the innate immune system and consequent immunological profile.

Of note, in this Ugandan birth cohort, all participants were documented to have received BCG at birth, with the strain of BCG used recorded. This will therefore be the first well-powered study to investigate effects of BCG revaccination on vaccine responses in adolescents.
Open access

It will not investigate the effects of a first dose of BCG in adolescence.

For this work, all participants will receive BCG-Russia strain, provided by the Serum Institute of India. While responses to strains vary, this strain is widely available globally and in use in Uganda. For comparability, it will be used across the three trials, POPVAC A, POPVAC B and POPVAC C. In the context of these trials, it will not be possible to determine whether different strains of BCG would have different effects on other vaccines.

This study will determine whether BCG immunisation alters the response to live or inert vaccines given after 4 weeks, including effects on vaccine replication, immune response profile, priming, boosting and waning among adolescents who received BCG as infants. It will indicate whether including BCG as a component of school-based immunisation schedules is likely to have non-specific benefits for Ugandan adolescents and other settings where infant BCG immunisation is common. If this is correct, BCG immunisation may be used as a tool for inducing enhanced benefits for other vaccines in a wide range of settings.

Study timeline

Applications for ethical approval were submitted in May 2018, with approval received in September 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug Authority and Uganda National Council for Science and Technology) and June 2019 (London School of Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings were also held during the initial 12-month planning period. Recruitment is scheduled to commence in May 2020. Intervention will be up to 12 months, with completion of the project scheduled for April 2022.

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Contributors

AME conceived the study. AME, GN, EW, AN, AW, SC, LZ and MM contributed to study design. LZ, GO, GK, JS, CO, MN, EN, FA and JT are site clinicians/nurses/clinical laboratory technicians providing valuable input on clinical considerations of the intervention. MS, SK, FK, RK and MK are field workers and administrators handling the organisational integration of the intervention. AN, AM, HA and EW are involved in organisation of the databases, trial randomisation, treatment allocation and drawing up of analytical plans. LZ, GN, JN, AN, SC, EW and AME drafted the manuscript. All authors reviewed the manuscript, contributed to it and approved the final version.

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Competing interests

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Supplemental material

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REFERENCES

1 Benn CS, Netea MG, Selin LK, et al. A small jab — a big effect: nonspecific immunomodulation by vaccines. Trends Immunol 2013;34:431–9.
2 Rieckmann A, Villumsen M, Sorup S, et al. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. Int J Epidemiol 2017;46:695–705.
3 Prentice S, Webb EL, Dockrell HM, et al. Investigating the non-specific effects of BCG vaccination on the innate immune system in Ugandan neonates: study protocol for a randomised controlled trial. Trials 2015;16:149.
4 Biering-Sorensen S, Aaby P, Lund N, et al. Early BCG-Denmark and Neonatal Mortality Among Infants Weighing <2500 g: A Randomized Controlled Trial. Clin Infect Dis 2017;65:1183–90.
5 Blok BA, Arts RJW, van Crel R, et al. Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines. J Leukoc Biol 2015;98:347–56.
6 Kleinnijenhuis J, Quintin J, Preijers F, et al. Bacille Calmette-Guerin induces NOD2-dependent non-specific protection from reinfecction via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci U S A* 2012;109:17537–42.

7 de Bree LCJ, Koeken V, Joosten LAB, et al. Non-specific effects of vaccines: current evidence and potential implications. *Semin Immunol* 2018;39:35–43.

8 van der Heijden C, Noz MP, Joosten LAB, et al. Epigenetics and trained immunity. *Antioxid Redox Signal* 2018;29:1023–40.

9 Rusek P, Wala M, Druszczyńska M, et al. Infectious agents as stimuli of trained innate immunity. *Int J Mol Sci* 2018;19. doi:10.3390/ijms19020456. [Epub ahead of print: 03 Feb 2018].

10 Schrum JE, Crabtree JN, Dobbs KR, et al. Cutting Edge: Plasmodium falciparum Induces Trained Innate Immunity. *J Immunol* 2013;158:200–7.

11 Dayal D, Gupta S. Connecting BCG vaccination and COVID-19: additional data. *medRxiv* 2020:20053272.

12 Miller A, Reandelar MJ, Fasciglione K, et al. Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study. *medRxiv* 2020.

13 Leentjens J, Kox M, Stokman R, et al. Bcg vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study. *J Infect Dis* 2015;212:1930–8.

14 Arts RJW, Moorlag SJCFM, Novakov B, et al. Bcg vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. *Cell Host Microbe* 2016;23:99–100.

15 Webb EL, Mawa PA, Ndibazza J, et al. Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011;377:52–62.

16 Barreto ML, Piñeiro D, Pereira SM, et al. Causes of variation in BCG vaccine efficacy: examining evidence from the BCG REVAC cluster randomized trial to explore the masking and the blocking hypotheses. *Vaccine* 2014;32:3759–64.

17 Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.

18 Harris PA, Taylor R, Minor BL, et al. The REDCap Consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.

19 Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.

20 Muyanja E, Ssemaganda A, Ngauv P, et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. *J Clin Invest* 2014;124:3147–58.

21 Akondy RS, Johnson PLF, Nakaya H, et al. Initial viral load determines the magnitude of the human CD8 T cell response to yellow fever vaccination. *Proc Natl Acad Sci U S A* 2015;112:3050–5.

22 Sabin EA, Araujo MI, Carvalho EM, et al. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with Schistosoma mansoni. *J Infect Dis* 1996;173:269–72.

23 Elliott AM, Mawa PA, Webb EL, et al. Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to BCG and tetanus immunisation. *Vaccine* 2010;29:247–55.

24 Brown J, Baisley K, Kavishe B, et al. Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania. *Vaccine* 2014;32:611–7.

25 Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol* 2010;17:1055–65.

26 Riner DK, Ndombi EM, Carter JM, et al. Schistosoma mansoni infection can jeopardize the duration of protective levels of antibody responses to immunizations against hepatitis B and tetanus toxoid. *PLoS Negl Trop Dis* 2016;10:e0005180.

27 Fletcher HA, Snowden MA, Landry B, et al. T-cell activation is an immune correlate of risk in BCG vaccinated infants. *Nat Commun* 2016;7:11290.

28 Safaeian M, Porras C, Pan Y, et al. Durable antibody responses following one dose of the bivalent human papillomavirus L1 virus-like particle vaccine in the Costa Rica vaccine trial. *Cancer Prev Res* 2013;6:1242–50.

29 WHO. Immunization, vaccines and biologicals. vaccine position papers, 2017.

30 Aaby P, Benn C, Nielsen J, et al. Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. *BMJ Open* 2012;2:e000707.

31 Krishnan A, Srivastava R, Dwivedi P, et al. Non-specific sex-differential effect of DTP vaccination may partially explain the excess girl child mortality in Ballabgarh, India. *Trop Med Int Health* 2013;18:1329–37.