

BMJ Open 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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For numbered affiliations see end of article.

Correspondence to

Dr Gyaviira Nkurunungi;
Gyaviira.Nkurunungi@
mrcuganda.org

Ludoviko Zirimenya,¹ Gyaviira Nkurunungi ¹, Jacent Nassuuna,¹ Agnes Natukunda,¹ Alex Mutebe,¹ Gloria Oduru,¹ Grace Kabami,¹ Hellen Akurut,¹ Caroline Onen,¹ Milly Namutebi,¹ Joel Serubanja,¹ Esther Nakazibwe,¹ Florence Akello,¹ Josephine Tumusiime,¹ Moses Sewankambo,¹ Samuel Kiwanuka,¹ Fred Kiwudhu,¹ Robert Kizindo,¹ Moses Kizza,¹ Anne Wajja,¹ Stephen Cose,^{1,2} Moses Muwanga,³ Emily Webb,⁴ Alison M Elliott,^{1,2} The POPVAC trial team

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.^{1–4} 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.^{5–8} This process, by which the innate immune system develops a form of memory, has been called ‘trained innate immunity’.⁹ Evidence that a range of stimuli, including bacterial products (particularly *Salmonella typhi* lipopolysaccharide (LPS)), and infections, including malaria and hepatitis B,¹⁰ 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 life⁹ 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.^{11 12}

In Europe, BCG vaccination 2 weeks before influenza vaccination has been shown to result in enhanced antibody responses to influenza proteins.¹³ 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.¹⁴

In Uganda, BCG immunisation at birth is recommended.¹⁵ The benefits of BCG immunisation in adolescence for protection against tuberculosis are not known and may differ between settings.¹⁶ 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 cohort¹⁵ 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

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Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting guidelines¹⁷ 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.¹⁵ In EMaBS, a cohort of 2500 pregnant women were recruited between 2003 and 2005 for a trial of antelmintic treatment during pregnancy and early childhood, investigating effects on childhood vaccine responses and infectious disease incidence.¹⁵ 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.¹⁵
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 ≥ 5 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.^{18 19} 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,^{20 21} but bias, or even enhance, responses to toxoids or proteins²²⁻²⁴; 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.

**Table 1** Immunisation schedule

	Immunisation week 0	Immunisation week 4	(Immunisation week 8)	Immunisation week 28	(Immunisation week 52)
Live vaccines	BCG revaccination*	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime	HPV boost for girls aged ≥ 14 years ^{†,‡}	HPV boost and Td boost	Td boost ^{‡,§}

*Prior BCG status may vary (data on history and documentation of prior BCG, and presence of a BCG scar, will be documented although these approaches have limitations for determining BCG status).

[†]The National Expanded Programme on Immunisation recommends three doses of HPV vaccine for older girls.

[‡]These doses will be given to comply with guidelines, but outcomes specifically relating to these doses will not be assessed.

[§]Priming by immunisation in infancy is assumed.

HPV, human papillomavirus; Td, tetanus/diphtheria.

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)²⁵ 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,²⁶ 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^{20 27 28} and preliminary data, we anticipate that SDs of primary outcome measures will lie between 0.3 and 0.6 log₁₀, and that revaccination with BCG may increase responses by approximately 0.12–0.14 log₁₀. Based on these assumptions, we aim to enrol 300 EMaBS participants (150 BCG revaccination, 150 no BCG revaccination). Allowing for 10% loss to follow-up, this will give over 90% power to detect a difference of 0.12 log₁₀ 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 log₁₀ (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: CTA0094). 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

Table 2 Power estimates (5% significance level)

SD (log ₁₀)	Log ₁₀ difference						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
Trial C: 150 BCG immunisation vs 150 no BCG immunisation							
0.3	59%	78%	91%	97%	99%	>99%	>99%
0.4	37%	53%	69%	82%	91%	96%	98%
0.5	26%	37%	50%	63%	75%	84%	91%
0.6	19%	28%	37%	48%	59%	69%	78%

Cells highlighted in grey correspond to >80% power.

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 2017,²⁹ 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,^{18 19} 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).^{1 2} 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.^{30 31} It has been further suggested that reducing time of exposure to DTP as the most recent vaccination with BCG may reduce this childhood mortality.³⁰

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.¹⁵ This will therefore be the first well-powered study to investigate effects of BCG revaccination on vaccine responses in adolescents.

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.

Author affiliations

¹Immunomodulation and Vaccines Programme, MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Wakiso, Uganda

²Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK

³Entebbe Hospital, Entebbe, Wakiso, Uganda

⁴MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, UK

Twitter Gyaviira Nkurunungi @GyavN

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Collaborators POPVAC trial team: Principal investigator: Alison Elliott; Project leader: Ludoviko Zirimenya; laboratory staff: Gyaviira Nkurunungi, Stephen Cose, Rebecca Amongin, Beatrice Nassanga, Jacent Nassuuna, Irene Nambuya, Prossy Kabuubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami; statisticians and data managers: Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; clinicians: Anne Wajja, Milly Namutebi, Christopher Zziwa, Joel Serubanja; nurses: Caroline Onen, Esther Nakazibwe, Josephine Tumusiime, Caroline Ninsiima, Susan Amongi, Florence Akello; internal monitor: Mirriam Akello; field workers: Robert Kizindo,

Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred Kiwudhu; boatman: David Abiriga; administrative management: Moses Kizza, Samsi Nansukusa; internal and external collaborators: Pontiano Kaleebu, Hermelijin Smits, Maria Yazdanbakhsh, Govert van Dam, Paul Corstjens, Sarah Staedke, Henry Luzze, James Kaweesa, Edridah Tukahebwa, Elly Tumushabe, Moses Muwanga.

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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ORCID iD

Gyaviira Nkurunungi <http://orcid.org/0000-0003-4062-9105>

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1 [SUPPLEMENTARY INFORMATION](#)

2

3 **The impact of Bacillus Calmette-Guérin revaccination on the response to unrelated vaccines in a**
4 **Ugandan adolescent birth cohort: randomised controlled trial protocol C for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**

6 Ludoviko Zirimenya^{1,¶}, Gyaviira Nkurunungi^{1,¶,*}, Jacent Nassuuna^{1,¶}, Agnes Natukunda^{1,¶}, Alex
7 Mutebe¹, Gloria Oduru¹, Grace Kabami¹, Hellen Akurut¹, Caroline Onen¹, Milly Namutebi¹, Joel
8 Serubanja¹, Esther Nakazibwe¹, Florence Akello¹, Josephine Tumusiime¹, Moses Sewankambo¹,
9 Samuel Kiwanuka¹, Fred Kiwudhu¹, Robert Kizindo¹, Anne Wajja¹, Stephen Cose^{1,2}, Moses Muwanga³,
10 Emily L Webb⁴, Alison M Elliott^{1,2} **for the POPVAC trial team**

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12 ¹Immunomodulation and Vaccines Programme, Medical Research Council/Uganda Virus Research
13 Institute and London School of Hygiene and Tropical Medicine (MRC/UVRI and LSHTM) Uganda
14 Research Unit, Entebbe, Uganda

15 ²Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, United
16 Kingdom

17 ³Entebbe Hospital, Entebbe, Uganda

18 ⁴MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London School
19 of Hygiene and Tropical Medicine, London, United Kingdom

20 [¶]These authors contributed equally

21 ***Correspondence:** Gyaviira Nkurunungi; Gyaviira.Nkurunungi@mrcuganda.org

22 Table S1: Schedule of visits and procedures

VISIT NUMBER	1	2	3	3.2, 3.3	4	5	6
WEEKS FROM 1 ST IMMUNISATION	-4 to 0 ¹	0	4	4 weeks +4 days	8	28	52 SE
	Screening	Immunisation	Immunisations		Primary endpoint (PE)	Immunisations	Secondary endpoint (SE)
RANDOMISED BCG "IMMUNISATION"							
BCG arm (x)		x					
No BCG arm (o)		o					
ANTHELMINTHIC TREATMENT							
Praziquantel and albendazole or mebendazole					X ²	X ²	X ²
VACCINES							
YF-17D			x				
Ty21a			X ⁶				
HPV			x		[x] ⁴	x	
Td						x	[x] ⁵
INVESTIGATIONS/PROCEDURES							
Inclusion/exclusion criteria	x						
Informed consent	x						
Questionnaire	x		x	x	x	x	x
Examination	x		(x)	(x)	(x)	(x)	(x)
Urine β-HCG test (female only) 1mL	x	X ⁵	x			x	
Urine YF viral load				x			
Stool for PCR and storage	x						x
Stool for coproantibody and storage	x				x		
BLOOD TESTS							
Malaria PCR (1ml)	x						x
Serology for HIV, prior malaria and <i>S. mansoni</i> (0.5 ml)	x						
Mansonella perstans (1ml)	x						
Full blood count (1ml)	x		x				
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁹ (10-20ml)	x		x		x		x
Blood for gene expression (2ml)	x		x				
Blood vol (mL)	27		17		20		25
Cumulative blood vol (mL) ⁸	27		44		64		89
<p>PE: primary endpoint; SE: secondary endpoint Immunisation days highlighted in green, primary end point days in red (x) performed if clinically indicated</p> <p>1. Screening and enrolment into Project C will take place shortly before enrolment, sometimes on the same day</p>							

2. Treatments given after sample when schedules coincide
3. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years
4. Week 52 Td booster dose will be provided as a service
5. Pregnancy test to be repeated if more than 4 weeks elapses between screening and immunisation
6. Oral typhoid vaccine doses will be administered on three alternate days namely visit 3, 3.1, and 3.2
7. Exploratory immunology blood volume will be guided by guidelines from Harvard Mass General, where a maximum of 3ml/kg body weight is taken at any one time point and not more than 3ml/kg is taken over any 8-week period (ref http://www.drgrgreen.com/21_1616.html.) These guidelines have been followed in a previous study vaccinating adolescents with investigational tuberculosis vaccine MVA85A (in Uganda).¹ The total blood volume planned is 64 ml over the initial intensive sampling period of 8 weeks. Revision of sample volumes based on weight will only be required for participants who weigh less than 21 kg; the average weight of children aged 9 years is expected to be 28kg (with 21kg the 3rd centile) with greater weights for older children.²

24 **Further rationale for the selection of vaccines**

25 *Yellow fever vaccine*

26 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
27 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
28 wider region³ and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI; H
29 Luzze, personal communication). As noted above, lower vaccine replication, lower neutralising
30 antibody induction, and greater waning, are described in Uganda compared to Switzerland.⁴ YF-17D
31 is a potential vector for novel vaccine constructs,⁵ adding relevance to vaccine development.

32 *Typhoid vaccine Ty21a*

33 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
34 constructs.⁶ Ty21a vaccine will be purchased from PaxVax, Redwood City, California. Substantial,
35 multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been advocated as
36 cost effective.⁷

37 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
38 currently) registered in many countries. It was first registered in the United States and United
39 Kingdom in the 1980s, and is recommended by the World Health Organisation for both endemic and
40 epidemic settings.⁸ It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine,
41 good durability and minimal adverse effects.⁸ It is proposed for use in this study to model effects of
42 study exposures and intervention on the response to a live oral vaccine.

43 The Ty21a vaccine is given as a three-dose regimen on alternate days.

44 *Human Papilloma Virus (HPV) vaccine*

45 Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV Vaccine
46 Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national EPI
47 programme. HPV immunisation is being rolled out among girls to prevent cervical neoplasia, the
48 commonest cancer among Ugandan women and we will coordinate provision with the national HPV
49 immunisation programme.⁹ HPV immunisation is also beneficial for boys since HPV infection is
50 associated with anogenital warts, anal cancer and oropharyngeal cancers in both males and females,
51 and with penile cancer in men,¹⁰ and we will include boys in these studies.

52 *Tetanus and diphtheria vaccines*

53 Tetanus and diphtheria vaccines comprise inert toxoids (Td). Booster immunisation is recommended
54 for young women to prevent maternal and neonatal tetanus. Recent evidence emphasises the need
55 to protect young men also.¹¹

56 **Immunisation Postponement Criteria**

57 If any one of the following is identified at the time scheduled for immunisation, the participant may
58 be immunised at a later date, or withdrawn, at the discretion of the Investigator. The participant
59 must be followed until resolution of the event as with any adverse event:

- 60 • Acute disease at the time of immunisation. Acute disease is defined as the presence of a
61 moderate or severe illness with or without fever. All vaccines can be administered to
62 persons with a minor illness such as diarrhoea or mild upper respiratory infection with or
63 without low-grade fever, i.e. temperature of $\leq 37.5^{\circ}\text{C}$ (99.5°F)
- 64 • Temperature of $>37.5^{\circ}\text{C}$ (99.5°F) at the time of immunisation
- 65 • Taking antibiotics or antimalarials currently, or within the past 7 days, of the date of Ty21a
66 administration (ascertained verbally)

67 **Vaccine storage and transport**

68 In order to maintain a reliable vaccine cold chain, the vaccines and diluents to be used will be stored
69 and transported within the recommended temperature range of $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. Care will be taken to
70 ensure that the vaccines are not frozen. BCG, being sensitive to light, will be kept in the dark (normally
71 within its secondary packaging) for as long as possible to protect it during storage and transportation.
72 All vaccines will be kept in appropriate refrigeration equipment with a temperature monitoring device
73 to ensure temperatures remain between $+2^{\circ}\text{C}$ and $+8^{\circ}\text{C}$. Cold boxes/vaccines carriers with
74 temperature monitors will be used to transport vaccines and the diluents from the MRC/UVRI and
75 LSHTM Uganda Research Unit (Entebbe) to the clinic where vaccination will take place and while
76 transporting vaccines to immunisation sessions. Designated staff will be given responsibility for
77 managing the vaccine cold chain. All cold chain equipment including the temperature monitoring
78 devices used for this project will comply with relevant technical specifications as defined by the EPI
79 standards. Basic routine maintenance will be regularly carried out on all cold chain equipment.

80 **Additional laboratory measurements**

81 Additional assays will comprise measurement of parasite infection exposure, HIV serology, pregnancy
82 testing and full blood counts. HIV testing and pregnancy testing will be accompanied by appropriate
83 counselling by trained staff.

84 **Current *S. mansoni* infection status and intensity** will be determined by serum/plasma levels of
85 circulating anodic antigen (CAA). The method is quantitative, highly specific for *Schistosoma* infection,
86 and much more sensitive than the conventional Kato Katz method.¹² CAA will be assessed
87 retrospectively on stored samples collected at baseline.

88 **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg antigen
89 using stored blood samples collected at baseline.

90 **The presence of other helminth infections** will be determined retrospectively using stool PCR of
91 samples collected at baseline and at weeks 28 and 52.¹³ In accordance with national guidelines, all
92 participants will be treated with albendazole or mebendazole after collection of samples for primary
93 endpoints at week 8 and 28, and after collection of samples for secondary endpoints at week 52.

94 **Current malaria infection status and intensity** will be assessed retrospectively by PCR on stored
95 samples collected on immunisation days and at week 52.

96 **Malarial fever:** Individuals presenting with fever will be investigated using rapid diagnostic tests for
97 malaria and treated based on the results and according to prevailing national guidelines.

98 **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored samples
99 collected at baseline.

100 **HIV serology** will be done on blood samples using rapid tests and according to prevailing national
101 algorithms. The current algorithm is shown in Appendix 2. This will be done at baseline.

102 **Pregnancy testing** will be done using urine samples and standard operating procedures for
103 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline and
104 before immunisation on each immunisation day.

105 **Full blood counts** will be conducted using a haematology analyser. Mild, moderate and severe
106 anaemia will be defined according to WHO guidelines, by age.¹⁴ This will be done at baseline (to test
107 for anaemia as part of the eligibility assessment), and pre-immunisation as part of the assessment of
108 immunological profile.

109 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
110 care.

111 Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised
112 intervention (since the intervention might be beneficial in management of anaemia). They will be
113 treated for anaemia and for any underlying cause identified.

114 **Operational considerations**

115 *Programme governance*

116 A Programme Steering Committee will be set up to guide progress across all projects. This will
117 comprise the following:

- 118 • An independent chair
- 119 • Representatives from the Ministry of Health programmes for immunisation and for vector
- 120 borne disease control
- 121 • Representatives of district authorities (Mukono and Jinja districts)
- 122 • Community representatives
- 123 • Principal investigator and co-investigators
- 124 • Project leader and post-doctoral immunologist
- 125 • Trial statistician
- 126 • Laboratory manager
- 127 • Medical Research Council observer

128 *Informed consent*

129 Both written informed assent from the participants and written informed consent from a parent or
130 guardian will be required for participation, although these may not necessarily be obtained at the
131 same time. Information will be provided in both English and the appropriate local language. For
132 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
133 read the information sheet and translate the information to the participant or parent/guardian will
134 be used. Informed consent by emancipated or mature minors will be obtained using designated
135 consent form for these kinds of participants.

136 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
137 will be explained. The participant will be given the opportunity to ask about details of the trial, and
138 will then have time to consider whether or not to participate. If they do decide to participate, they
139 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
140 them to take away and keep, and one to be stored securely by the research team. Separate
141 information and consent forms will be provided for consent for storage of samples for future studies
142 and for anonymous sharing of data from this study. For the EMaBS cohort genetic data are already
143 available based on previous approval; the information sheet will explain that these data may be used
144 in analyses related to this protocol.

145 *Screening and Eligibility Assessment*

146 Once the informed consent process has been completed, and consent (and assent) given, a baseline
147 medical history (including concomitant medication) will be collected. Vital signs will be checked and
148 a physical examination will be performed. Inclusion and exclusion criteria will be checked.

149 Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a
150 trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be
151 obtained, for tests as specified in the schedule of procedures (Appendices A-C). These tests are to
152 exclude the major, immunomodulating co-infection, HIV, and conditions that might impact safety
153 (anaemia, pregnancy).

154 *Enrolment*

155 Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria
156 and meet none of the exclusion criteria will be enrolled into the trial. On the enrolment day (which
157 may be the same as the screening day in some cases) eligibility will be checked and participants will
158 be enrolled sequentially to the next randomisation number. They will then be given BCG vaccine or
159 not, according to their allocation.

160 *Discontinuation / withdrawal criteria*

161 In accordance with the principles of the current revision of the Declaration of Helsinki and any other
162 applicable regulations, a participant has the right to withdraw from the study at any time and for any
163 reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the
164 participant at any time in the interests of the participant's health and well-being. In addition, the
165 participant may withdraw/be withdrawn for any of the following reasons:

- 166 • Ineligibility (either arising during the study or retrospectively, having been overlooked at
167 screening)
- 168 • Administrative decision by the Investigator
- 169 • Significant protocol deviation
- 170 • Participant non-compliance with study requirements
- 171 • An adverse event which requires discontinuation of the study involvement or results in
172 inability to continue to comply with study procedures.

173 Any participant who becomes pregnant during the trial will be followed up until the end of the
174 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
175 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
176 will only be given further treatment if clinically indicated. The babies will also be followed up and
177 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
178 participant.

179 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
180 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
181 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

182 If a participant withdraws from the study samples collected before their withdrawal from the trial
183 will be used/ stored unless the participant specifically requests otherwise.

184 *Trial discontinuation*

185 The Trial will be discontinued in the event of new scientific information that renders continuation
186 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

187 *End of study definition*

188 The trial will be completed when the last participant enrolled into the trial has completed their final
189 follow up visit.

190 *Safety assessments and oversight*

191 No new investigational drug or product will be used in the proposed trial. However, standard
192 approaches for monitoring safety and reporting of serious adverse events will be followed.

193 *Monitoring*

194 The trial will be monitored by both internal and external monitors according to a pre-defined
195 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
196 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
197 and to Good Clinical Research Practice procedures.

198 ***Procedures to be followed in the event of abnormal findings***

199 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
200 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
201 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
202 and appropriate medical care arranged as appropriate and with the permission of the participant.
203 Specific details regarding findings, discussion with participants and resulting actions will be recorded
204 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
205 a participant from the trial will be at the discretion of the Investigator.

206 **Data and Safety Monitoring Board (DSMB)**

207 A data and safety monitoring board (DSMB) will be appointed to provide real-time safety oversight.
208 The DSMB will be notified within 7 days of the Investigators' being aware of the occurrence of SAEs.
209 The DSMB may recommend the Investigators to place the trial on hold if deemed necessary
210 following an intervention-related SAE. The DSMB will be chaired by a clinician experienced in clinical
211 trials. There will be a minimum of two other appropriately qualified committee members. In the case
212 of events related to a blinded intervention, the DSMB can request unblinding. Membership will
213 include a statistician, and at least one Ugandan member. All correspondence between Investigators
214 and the DSMB will be conveyed by the Principal Investigator to the trial Sponsor. The Chair of the
215 DSMB will be contacted for advice and independent review by the Investigator or trial Sponsor in the
216 following situations:

- 217 • The occurrence of any SAE
- 218 • Any other situation where the Investigator or trial Sponsor feels independent advice or
219 review is important.

220 **Ethical and regulatory considerations**

221 *Information regarding risks and benefits to the participant*

222 Participants in this programme will be adolescents and therefore a vulnerable human population.
223 Care will be taken to provide adequate, age and education-status appropriate information and to
224 ensure that it is understood; and to emphasise that participation is voluntary. Participants will be
225 enrolled only when they have given their own assent and when consent has been given by the
226 parent or guardian.

227 No major risks to the participants are anticipated since all the treatments and vaccines to be given
228 are licensed and known to be safe. The main risk to participants will be time lost from school work:
229 we will work with parents to minimise disruption to studies.

230 Participants will suffer the discomfort and inconvenience of providing blood samples (and stool and
231 urine samples). Occasionally people faint when a vaccine is given or when blood is drawn.
232 Individuals will be comfortably seated during these procedures and the research team will be trained
233 to manage such events.

234 The immunisations to be given have recognised side effects which are usually mild and resolve
235 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
236 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
237 associated with difficulty in moving the shoulder. Sometimes headache and tiredness occurs. Rarely

238 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
239 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).¹⁵ Individuals with a history of a
240 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
241 proteins, will be excluded from the studies. The research team will be trained and prepared to
242 manage severe allergic reactions.

243 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
244 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
245 mortality for this severe, life-threatening adverse effect is reported as about 50%.¹⁵

246 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
247 starting as a small papule at the injection site which may become ulcerated and then heal over a
248 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
249 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
250 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
251 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
252 occurs in immunocompromised people: HIV positive people will be excluded from these studies.¹⁶
253 BCG “pre-immunisation” may interfere with the response to the subsequent live vaccines; indeed
254 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.¹⁷
255 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
256 of neutralising antibody titres (which are the desired protective outcome).^{4,17}

257 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
258 and (rarely) rash.¹⁵

259 **Benefits**

260 All the vaccines to be given are licensed and regarded as safe. In general, the vaccines and
261 treatments are expected to provide protection against infectious diseases. Participants and their
262 families, and communities are expected to benefit from improved understanding of vaccines.

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