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The impact of Bacillus Calmette-Guérin “pre-immunisation” 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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3 1 **The impact of Bacillus Calmette-Guérin “pre-immunisation” on the response to unrelated vaccines**
4 **in a Ugandan adolescent birth cohort: randomised controlled trial protocol C for the ‘POPulation**
5 **differences in VACCine responses’ (POPVAC) programme**
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3 **20 Abstract**

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5 **21 Introduction**

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8 **22** There is evidence that Bacillus Calmette–Guérin (BCG) immunisation may protect against unrelated
9 **23** infectious illnesses. This has led to the postulation that administering BCG before unrelated vaccines
10 **24** may enhance responses to these vaccines. This might also model effects of BCG on unrelated
11 **25** infections.

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14 **26 Methods and analysis**

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16
17 **27** To test this hypothesis, we have designed a randomised controlled trial of BCG versus no BCG
18 **28** immunisation to determine the effect of BCG on subsequent unrelated vaccines, among 300
19 **29** adolescents (ages 13 to 17 years) from a Ugandan birth cohort. Our schedule will comprise three
20 **30** main immunisation days (week 0, week 4 and week 28): BCG (or no BCG) pre-immunisation at week
21 **31** 0, Yellow fever (YF-17D), Oral typhoid (Ty21a) and HPV prime at week 4, HPV boost and
22 **32** Tetanus/diphtheria (Td) boost at week 28. Primary outcomes are anti-YF-17D neutralising antibody
23 **33** titres, *Salmonella typhi* lipopolysaccharide (LPS)-specific IgG concentration, IgG specific for L1-
24 **34** proteins of HPV-16/18 and tetanus and diphtheria toxoid-specific IgG concentration, all assessed at
25 **35** four weeks after immunisation with YF, Ty21a, HPV and Td, respectively. Secondary analyses will
26 **36** determine effects on correlates of protective immunity (where recognised correlates exist), on
27 **37** vaccine response waning and on whether there are differential effects on priming vs boosting
28 **38** immunisations. We will also conduct exploratory immunology assays among subsets of participants
29 **39** to further characterise effects of BCG pre-immunisation on vaccine responses. Further analyses will
30 **40** assess which life-course exposures influence vaccine responses in adolescence.

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33 **41 Ethics and dissemination**

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36 **42** Ethics approval has been obtained from relevant Ugandan and UK ethics committees. Results will be
37 **43** shared with Uganda Ministry of Health, relevant district councils, community leaders and study
38 **44** participants. Further dissemination will be done through conference proceedings and publications.

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41 **45 Trial registration**

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44 **46** Current Controlled Trials identifier: ISRCTN10482904

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47 **47**

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49 **48 Article summary**

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52 **49** *Strengths and limitations of this study*

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3 50 • This will be the first well-powered trial to investigate effects of BCG pre-immunisation on
4
5 51 responses to unrelated vaccines in adolescents.
6
7 52 • Effects on both live-attenuated and inert vaccines will be studied.
8
9 53 • Our robust immunoepidemiological design and nested immunological studies will address
10 54 specific hypotheses regarding pathways of effects of BCG pre-immunisation on unrelated
11 55 vaccine responses.
12
13 56 • One limitation is that interaction between the three vaccines administered together one
14 57 month after BCG immunisation may mask the true effect of BCG pre-immunisation on
15 58 individual vaccine responses.
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21 60 **Word count**

22 61 2780

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24 62 **Keywords**

25 63 Vaccine; BCG; Immunization; Uganda
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64 Introduction

65 There is increasing evidence that *Bacillus Calmette–Guérin* (BCG) immunisation has non-specific,
66 protective effects relating to infections other than tuberculosis.¹⁻³ Experimental studies using BCG
67 suggest that effects on the innate immune response are an important component of this
68 phenomenon: BCG immunisation induces lasting epigenetic modification of innate immune cells,
69 including monocytes, macrophages and natural killer cells.^{4,5} This process, by which the innate
70 immune system develops a form of memory, has been called “trained innate immunity”.⁶ Evidence is
71 accumulating that a range of stimuli including bacterial products (particularly *Salmonella typhi*
72 lipopolysaccharide (LPS)), and infections including malaria and hepatitis B,⁷ may induce trained
73 innate immunity; that the profile into which cells are trained varies with the dose and characteristics
74 of the stimulus; and that effects may be induced prenatally (on exposure to maternal infections) as
75 well as later in life.⁶

76 It is plausible that variation in the intensity and spectrum of experience of previous infections, and
77 hence the epigenetic programming and consequent functional profiles of innate immune cells,
78 contributes to the many differences in immunological activity observed between geographically and
79 environmentally distinct settings, and hence to differences in vaccine response. If this hypothesis is
80 correct, BCG immunisation can act as a model for the effects of prior infection, and may also be a
81 tool for inducing enhanced benefits for other vaccines. Vaccine-specific responses can also act as a
82 model for responses to infection. This is especially relevant given the current interest in the
83 potential benefit of BCG immunisation against COVID-19 disease.^{8,9}

84 In Europe, BCG “pre-immunisation” two weeks before giving influenza vaccine has been shown to
85 result in enhanced antibody responses to influenza proteins.¹⁰ BCG “pre-immunisation” four weeks
86 before giving Yellow Fever (YF 17D) vaccine has also been found to result in reduced replication of
87 the yellow fever vaccine virus; this was not associated with a significant reduction in the desired
88 neutralising antibody response to YF, or in the interferon (IFN)- γ response, but the study size was
89 small and may not have had sufficient power to demonstrate important effects.¹¹

90 In Uganda, BCG immunisation at birth is recommended.¹² The benefits of BCG immunisation in
91 adolescence for protection against tuberculosis are not known and may differ between settings.¹³
92 Whether BCG immunisation in adolescents in Uganda will have non-specific effects on the innate
93 immune response, on subsequent immunisations and (indeed) on general health (given the prior
94 exposure at birth, and the on-going exposure to non-tuberculous mycobacteria and other infections)
95 is not known. In Protocol C of the ‘Population differences in Vaccine responses’ programme

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3 96 (POPVAC C; Current Controlled Trials identifier: ISRCTN10482904), we plan to address this
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5 97 knowledge gap by randomising adolescent members of the Entebbe Mother and Baby Study
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7 98 (EMaBS) birth cohort¹² in a nested trial of BCG “pre-immunisation” versus no BCG immunisation
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9 99 prior to immunisation with other vaccines. We summarise the protocol here.
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3 100 **Hypothesis**
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5 101 The overarching goal of the POPVAC programme is to understand population differences in vaccine
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7 102 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
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9 103 optimised for the low-income, tropical settings where they are especially needed. For this Trial C we
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11 104 address the concept of trained innate immunity through the hypothesis that BCG “pre-
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13 105 immunisation” modifies the response to subsequent unrelated vaccines.

14 106 **Objective**
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16 107 To determine whether BCG “pre-immunisation” modulates the response to unrelated vaccines
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18 108 among Ugandan adolescents.
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3 109 **Methods and analysis**

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5 110 ***Setting and participants***

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7 111 SPIRIT reporting guidelines¹⁴ are used. This trial will be a randomised, controlled, open, parallel
8 112 group trial investigating the effect of BCG “pre-immunisation” on unrelated vaccine response
9 113 outcomes. The study will take place in Entebbe municipality, Wakiso District, Uganda and will involve
10 114 participants in the EMaBS birth cohort.¹² In EMaBS, a cohort of 2500 pregnant women were
11 115 recruited between 2003 and 2005 for a trial of anthelmintic treatment during pregnancy and early
12 116 childhood, investigating effects on childhood vaccine responses and infectious disease incidence.¹²
13 117 We aim to enroll 300 of the EMaBS birth cohort participants, randomising 150 to each intervention
14 118 arm. All EMaBS participants received BCG at birth; hence current trial participants (in the BCG
15 119 intervention arm) will undergo revaccination. EMaBS participants are expected to be aged 13 to 17
16 120 during recruitment to this study.

17
18 121 ***Recruitment criteria***

19
20 122 *Inclusion criteria*

- 21
22 123 i. A participant in the Entebbe Mother and Baby Study¹²
23 124 ii. Written informed consent by parent or guardian
24 125 iii. Written informed assent by participant
25 126 iv. Willing to remain in the study area for the duration of the study
26 127 v. Willing to provide locator information and to be contacted during the course of the trial
27 128 vi. Females agree to avoid pregnancy for the duration of the trial
28 129 vii. Able and willing (in the investigator's opinion) to comply with all the study requirements

29
30 130 *Exclusion criteria*

- 31
32 131 i. Concurrent enrolment into another clinical trial
33 132 ii. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
34 133 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
35 134 neurological illness
36 135 iii. History of serious psychiatric condition or disorder
37 136 iv. Moderate or severe acute illness characterised by any of the following symptoms: fever,
38 137 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as determined by
39 138 the attending project clinician.

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3 139 v. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human
4 140 Papillomavirus (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria
5 141 vaccine (Td) at age ≥ 5 years
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8 142 vi. Concurrent oral or systemic steroid medication or the concurrent use of other
9 143 immunosuppressive agents within 2 months prior to enrolment
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11 144 vii. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
12 145 component of the study vaccines including egg or chicken proteins
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15 146 viii. Tendency to develop keloid scars
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17 147 ix. Positive HIV serology
18 148 x. Positive pregnancy test
19
20 149 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during
21 150 the trial period
22
23 151 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
24 152 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
25 153 planned use during the study period
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28 154 xiii. Administration of immunoglobulins and/or any blood products within the three months
29 155 preceding the planned trial immunisation date
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32 **Interventions**

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34 157 We will randomise participants to receive BCG or not to receive BCG, four weeks prior to
35 158 immunisation with a panel of licensed unrelated vaccines (discussed below). The adolescents in the
36 159 intervention arm will receive a dose of 0.1 ml in the deltoid region of the right upper arm.

40 **Randomisation and allocation to treatment arm**

41
42 161 An independent statistician will generate the randomisation code using a randomly permuted block
43 162 size. This code will be embedded as a web-based randomisation system in REDCap (Research
44 163 Electronic Data Capture) software.^{15 16} Randomisation to the two trial arms will be done in a 1:1
45 164 ratio. At enrolment, eligibility criteria will be checked and eligible participants will be allocated
46 165 sequentially to the next randomisation number, with the corresponding trial arm designated in
47 166 REDCap. The randomisation code will be kept securely by the trial statistician with a second copy
48 167 held by a data manager or statistician not otherwise involved in the trial at the MRC/UVRI and
49 168 LSHTM Uganda Research Unit.

56 **Blinding**

57
58 170 This trial will not be blinded to clinicians or participants since they will not participate in outcome
59 171 ascertainment and the expected development of a BCG scar makes blinding difficult. It is unlikely

172 that participants allocated to “no BCG” will seek this privately. Only laboratory personnel evaluating
 173 vaccine response outcomes will be unaware of BCG allocation so outcome ascertainment will not be
 174 biased through lack of blinding.

175 **Immunisations**

176 We anticipate that BCG pre-immunisation may have different effects on live and non-live, oral and
 177 parenteral, priming and boosting vaccines. Activated innate responses may kill live vaccines and
 178 suppress subsequent adaptive responses by this, or other, mechanisms,^{17 18} but bias, or even
 179 enhance, responses to toxoids or proteins;¹⁹⁻²¹ thus, results from a single-vaccine study would not
 180 be generalisable.

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

190

Table 1. Immunisation schedule

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime	HPV boost for girls aged ≥ 14 years ^{2,3}	HPV boost and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{3,4}
1. 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) 2. The National EPI programme recommends three doses of HPV vaccine for older girls 3. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 4. Priming by immunisation in infancy is assumed					

191 **Schedule of immunisation and sampling**

192 The schedule of immunisation and sampling is outlined in **Table S1**. While optimal timings for
193 outcome measures vary between vaccines, sampling at 8 weeks post BCG and 4 weeks post YF-17D,
194 Ty21a, HPV and Td is proposed for the primary endpoints, targeting the establishment of memory
195 responses and approximate peak of antibody responses. A secondary endpoint at one year will
196 assess waning. All analyses will take baseline measurements into account. Immunisation
197 postponement criteria are detailed in Supplementary information.

198 **Outcomes**

199 *Primary outcomes*

200 These will be assessed in all participants.

- 201 i. **YF-17D**: neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post
202 YF immunisation.
- 203 ii. **Ty21a**: *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G
204 concentration at four weeks post Ty21a immunisation.
- 205 iii. **HPV**: IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming
206 immunisation.
- 207 iv. **Td**: tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td
208 immunisation.

209 *Secondary outcomes*

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

- 213 i. **Protective immunity**: Proportions with protective neutralising antibody (YF); protective IgG
214 levels (TT);²² seroconversion rates (Ty21a) at four weeks post the corresponding
215 immunisation.
- 216 ii. **Response waning**: Primary outcome measures (all vaccines) repeated at week 52, and area-
217 under-the curve (AUC) analyses. Parasitic infection may accelerate,²³ and anti-parasitic
218 interventions delay, waning.
- 219 iii. **Priming versus boosting**: Effects on priming versus boosting will be examined for HPV only,
220 comparing outcomes four weeks after the first, and four weeks after the second vaccine
221 dose.

222 Furthermore, our sample collection will offer opportunities for an array of exploratory
 223 immunological evaluations on stored samples, focusing mainly on vaccine antigen specific outcomes.
 224 Exploratory assays will provide further detail on the mechanisms underlying effects of BCG on
 225 responses to unrelated vaccines.

226 *Additional measurements*

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

230 **Sample size considerations**

231 Based on the literature^{17 24 25} and preliminary data, we anticipate that standard deviations (SDs) of
 232 primary outcome measures will lie between 0.3 and 0.6 log₁₀; and that pre-immunisation with BCG
 233 may increase responses by approximately 0.12-0.14 log₁₀. Based on these assumptions, we aim to
 234 enrol 300 EMaBS participants (150 BCG “pre-immunisation”, 150 no BCG immunisation). Allowing
 235 for 10% loss to follow-up, this will give over 90% power to detect a difference of 0.12log₁₀ in vaccine
 236 response between the pre-BCG immunised and non-pre-immunised groups, at 5% significance level
 237 and assuming vaccine response standard deviation of 0.3log₁₀.

238

Table 2. Power estimates (5% significance level)

Standard deviation (log ₁₀)	Log ₁₀ difference						
	0.08	0.10	0.12	0.14	0.16	0.18	0.20
Trial C: 150 BCG “pre-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.

239

240 **Ethical and regulatory considerations**

241 Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus
 242 Research Institute (reference: GC/127/19/05/682), the London School of Hygiene and Tropical
 243 Medicine (reference: 16034), the Uganda National Council for Science and Technology (reference:
 244 HS 2491) and from the Uganda National Drug Authority (certificate number: CTA0094). Any protocol
 245 amendments will be submitted to ethics committees and regulatory bodies for approval before
 246 implementation.

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3 247 Participants will be adolescents and therefore a vulnerable human population. Care will be taken to
4
5 248 provide adequate, age- and education-status appropriate information and to ensure that it is
6
7 249 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
8
9 250 they have given their own assent and when consent has been given by the parent or guardian. No
10
11 251 major risks to the participants are anticipated since all the vaccines to be given are licensed and
12
13 252 known to be safe.

14 253 With regard to BCG immunisation or revaccination in adolescence, benefits with respect to
15
16 254 protection against tuberculosis among Ugandan adolescents are unknown and may, at best, be
17
18 255 modest. There may be non-specific benefits. WHO's SAGE committee concluded, in their summary
19
20 256 of October 2017,²⁶ that "BCG revaccination is safe in *Mycobacterium tuberculosis* infected and
21
22 257 uninfected populations. There is a lack of evidence from randomised controlled trials and
23
24 258 retrospective cohort and case-control studies demonstrating the efficacy and effectiveness of BCG
25
26 259 revaccination in adolescents and adults after primary BCG vaccination in infancy for protection
27
28 260 against TB disease. Due to absence of evidence, BCG revaccination is not considered cost-effective.
29
30 261 Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
31
32 262 *tuberculosis* exposure categories would benefit from BCG revaccination." We hope, through this
33
34 263 work, to contribute to this debate.

35 264 ***Patient and public involvement***

36 265 The EMaBS research team has previously worked with volunteer local council field workers to ensure
37
38 266 regular follow up of participants and these field workers continue to attend participants' meetings
39
40 267 and provide a mechanism by which the communities from which participants are drawn can be
41
42 268 informed about on-going work. As well, prior to the start of this study, we will share our plans with
43
44 269 district health and education officers, and with colleagues at Entebbe Hospital. We will establish an
45
46 270 advisory committee of parents who will help us to ensure that EMaBS cohort members can
47
48 271 participate in the study without undue disruption to their school work. Study findings will be shared
49
50 272 with these stakeholders and with participants.

51 273 ***Dissemination***

52 274 Study findings will be published through open access peer-reviewed journals, presentations at local,
53
54 275 national and international conferences and to the local community through community meetings.
55
56 276 Anonymised participant level datasets generated will be available upon request.

57 277 ***Data management and analysis***

58 278 Socio-demographic information and clinical and laboratory measurements will be recorded and
59
60 279 managed using REDCap (Research Electronic Data Capture) tools,^{15 16} with paper-based forms as

1
2
3 280 back-up. All data will be recorded under a unique study ID number. When paper forms must be
4
5 281 used, data will be double entered in a study-specific database, with standard checks for
6
7 282 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
8
9 283 protected server, with access limited to essential research personnel.

10 284 The effect of BCG versus no BCG pre-immunisation on the outcomes will be analysed. The analysis
11
12 285 will test whether BCG pre-immunisation alters the response to live or inert vaccines given four
13
14 286 weeks later, including effects on vaccine replication, immune response profile, priming, boosting and
15
16 287 waning. It will indicate whether including BCG as a component of school-based immunisation
17
18 288 schedules is likely to have non-specific benefits for Ugandan adolescents.

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289 Discussion

290 It is increasingly clear that several live vaccines, including BCG, measles vaccine and Vaccinia
291 (smallpox) vaccine, have non-specific, generally beneficial, effects including reduced mortality (not
292 related to the infectious disease that they were designed to target).^{1,2} The potential effects of BCG
293 on responses to unrelated vaccines, specifically on live-attenuated ones such as yellow fever and
294 oral typhoid, might model its effects on responses to unrelated infectious agents. We hypothesise
295 that BCG immunisation both achieves non-specific benefits, and influences vaccine responses,
296 through mechanisms based on effects on the innate immune system and consequent immunological
297 profile.

298 Of note, in this Ugandan birth cohort, all participants were documented to have received BCG at
299 birth, with the strain of BCG used recorded.¹² This will therefore be the first well-powered study to
300 investigate effects of BCG re-vaccination on vaccine responses in adolescents. This study will
301 determine whether BCG pre-immunisation alters the response to live or inert vaccines given four
302 weeks later, including effects on vaccine replication, immune response profile, priming, boosting and
303 waning among adolescents who received BCG as infants. It will indicate whether including BCG as a
304 component of school-based immunisation schedules is likely to have non-specific benefits for
305 Ugandan adolescents and other settings where infant BCG immunisation is common. If this is
306 correct, BCG immunisation may be used as a tool for inducing enhanced benefits for other vaccines
307 in a wide range of settings.

308

309 *Study timeline*

310 Applications for ethical approval were submitted in May 2018, with approval received in September
311 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
312 Authority and Uganda National Council for Science and Technology) and June 2019 (London School
313 of Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings
314 were also held during the initial 12-month planning period. Recruitment is scheduled to commence
315 in May 2020. Intervention will be up to 12 months, with completion of the project scheduled for
316 April 2022.

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3 317 **Competing interests**
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5 318 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
6

7 319 The rest of the authors declare that they have no conflicts of interest.
8

9 320 **Author contributions**
10

11 321 AME conceived the study. AME, GN, ELW, AN, AW, SC, LZ and MM contributed to study design. LZ,
12

13 322 GO, GK, JS, CO, MN, EN, FA and JT are site clinicians/nurses/clinical laboratory technicians providing
14

15 323 valuable input on clinical considerations of the intervention. MS, SK, FK, RK and MK are field workers
16

17 324 and administrators handling the organisational integration of the intervention. AN, AM, HA and ELW
18

19 325 are involved in organisation of the databases, trial randomization, treatment allocation and drawing
20

21 326 up of analytical plans. LZ, GN, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed
22

23 327 the manuscript, contributed to it and approved the final version.
24

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26

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28

29 330 for providing the HPV, yellow fever and oral typhoid vaccines, respectively. The BCG and tetanus-
30

31 331 diphtheria vaccines were kind donations from the Serum Institute of India. We thank the Wakiso
32

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34

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36

37 334 Monitoring Board (Dr David Meya, Prof. Andrew Prendergast and Dr Elizabeth George).
38

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42

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44

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46

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56

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58

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60

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3 347 The study sponsor (London School of Hygiene and Tropical Medicine) and funders had no role in
4
5 348 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
6
7 349 and the decision to submit the protocol for publication.
8
9 350

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11 351 **POPVAC trial team**

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13 352 **Principal investigator:** Alison Elliott; **Project leader:** Ludoviko Zirimenya; **laboratory staff:** Gyaviira
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15 353 Nkurunungi, Stephen Cose, Rebecca Amongin, Beatrice Nassanga, Jacent Nassuuna, Irene Nambuya,
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17 354 Prossy Kabuubi, Emmanuel Niwagaba, Gloria Oduru, Grace Kabami; **statisticians and data**
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19 355 **managers:** Emily Webb, Agnes Natukunda, Helen Akurut, Alex Mutebe; **clinicians:** Anne Wajja, Milly
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21 356 Namutebi, Christopher Zziwa, Joel Serubanja; **nurses:** Caroline Onen, Esther Nakazibwe, Josephine
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23 357 Tumusiime, Caroline Ninsiima, Susan Amongi, Florence Akello; **internal monitor:** Mirriam
24
25 358 Akello; **field workers:** Robert Kizindo, Moses Sewankambo, Denis Nsubuga, Samuel Kiwanuka, Fred
26
27 359 Kiwudhu; **boatman:** David Abiriga; **administrative management:** Moses Kizza, Samsi Nansukusa;
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29 360 **internal and external collaborators:** Pontiano Kaleebu, Hermelijn Smits, Maria Yazdanbakhsh,
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31 361 Govert van Dam, Paul Corstjens, Sarah Staedke, Henry Luzze, James Kaweesa, Edridah Tukahebwa,
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33 362 Elly Tumushabe, Moses Muwanga.
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3 1 [SUPPLEMENTARY INFORMATION](#)
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7 3 **The impact of Bacillus Calmette-Guérin “pre-immunisation” on the response to unrelated vaccines**
8 **in a Ugandan adolescent birth cohort: randomised controlled trial protocol C for the ‘POPulation**
9 **differences in VACCine responses’ (POPVAC) programme**
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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 "PRE-IMMUNISATION"							
BCG arm (x)		x					
No BCG arm (o)		o ²					
ANTHELMINTHIC TREATMENT							
Praziquantel and albendazole or mebendazole					X ³	X ³	X ³
VACCINES							
YF-17D			x				
Ty21a			X ⁷				
HPV			x		[x] ⁴	x	
Td						x	[x] ⁵
INVESTIGATIONS/PROCEDURES							
Inclusion/exclusion criteria	x						
Informed consent	x						
Questionnaire	x		[x] ²	x	x	x	x
Examination	x		(x)	(x)	(x)	(x)	(x)
Urine β-HCG test (female only) 1mL	x	x ⁶	[x] ²			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] ²				
Assessments of pre-immunisation responses, and/or vaccine response outcomes and/or exploratory immunology; storage ⁹ (10-20ml)	x		[x] ²		x		x
Blood for gene expression (2ml)	x		[x] ²				
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 1. Screening and enrolment into Project C will take place shortly before enrolment, sometimes on the same day</p>							

2. Individuals allocated to no BCG may be immunised immediately “skipping” to week 4. Procedures indicated in square brackets need not be repeated for the no BCG arm if this is done.
3. Treatments given after sample when schedules coincide
4. Week 8 HPV dose will be given for previously-unvaccinated girls aged ≥ 14 years
5. Week 52 Td booster dose will be provided as a service
6. Pregnancy test to be repeated if more than 4 weeks elapses between screening and immunisation
7. Oral typhoid vaccine doses will be administered on three alternate days namely visit 3, 3.1, and 3.2
8. 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.drgreene.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

23

For peer review only

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2
3 24 ***Further rationale for the selection of vaccines***
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5 25 *Yellow fever vaccine*
6

7 26 Yellow fever vaccine YF-17D is a live replicating parenteral vaccine. The vaccine (Stamaril; Sanofi
8 27 Pasteur) is available for purchase in Uganda. Yellow Fever (YF) causes outbreaks in Uganda and the
9 28 wider region³ and YF-17D is a candidate for Uganda's expanded programme on immunisation (EPI; H
10 29 Luzze, personal communication). As noted above, lower vaccine replication, lower neutralising
11 30 antibody induction, and greater waning, are described in Uganda compared to Switzerland.⁴ YF-17D
12 31 is a potential vector for novel vaccine constructs,⁵ adding relevance to vaccine development.

13 32 *Typhoid vaccine Ty21a*
14

15 33 Typhoid vaccine Ty21a is a live replicating oral vaccine and also a potential vector for new vaccine
16 34 constructs.⁶ Ty21a vaccine will be purchased from PaxVax, Redwood City, California. Substantial,
17 35 multi-year typhoid outbreaks occur in Uganda and immunisation campaigns have been advocated as
18 36 cost effective.⁷

19 37 Ty21a was developed in the 1970s. Although not routinely used in Uganda, it has been (and is
20 38 currently) registered in many countries. It was first registered in the United States and United
21 39 Kingdom in the 1980s, and is recommended by the World Health Organisation for both endemic and
22 40 epidemic settings.⁸ It has comparable efficacy to the parenteral Vi polysaccharide typhoid vaccine,
23 41 good durability and minimal adverse effects.⁸ It is proposed for use in this study to model effects of
24 42 study exposures and intervention on the response to a live oral vaccine.

25 43 The Ty21a vaccine is given as a three-dose regimen on alternate days.

26 44 *Human Papilloma Virus (HPV) vaccine*
27

28 45 Human Papilloma Virus (HPV) vaccine is a protein virus-like particle. The quadrivalent HPV Vaccine
29 46 Gardasil (Merck) is available for purchase in Uganda and is the vaccine used by the national EPI
30 47 programme. HPV immunisation is being rolled out among girls to prevent cervical neoplasia, the
31 48 commonest cancer among Ugandan women and we will coordinate provision with the national HPV
32 49 immunisation programme.⁹ HPV immunisation is also beneficial for boys since HPV infection is
33 50 associated with anogenital warts, anal cancer and oropharyngeal cancers in both males and females,
34 51 and with penile cancer in men,¹⁰ and we will include boys in these studies.

35 52 *Tetanus and diphtheria vaccines*
36

37 53 Tetanus and diphtheria vaccines comprise inert toxoids (Td). Booster immunisation is recommended
38 54 for young women to prevent maternal and neonatal tetanus. Recent evidence emphasises the need
39 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.

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3 88 **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg antigen
4
5 89 using stored blood samples collected at baseline.

6
7 90 **The presence of other helminth infections** will be determined retrospectively using stool PCR of
8
9 91 samples collected at baseline and at weeks 28 and 52.¹³ In accordance with national guidelines, all
10
11 92 participants will be treated with albendazole or mebendazole after collection of samples for primary
12
13 93 endpoints at week 8 and 28, and after collection of samples for secondary endpoints at week 52.

14
15 94 **Current malaria infection status and intensity** will be assessed retrospectively by PCR on stored
16
17 95 samples collected on immunisation days and at week 52.

18
19 96 **Malarial fever:** Individuals presenting with fever will be investigated using rapid diagnostic tests for
20
21 97 malaria and treated based on the results and according to prevailing national guidelines.

22
23 98 **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored samples
24
25 99 collected at baseline.

26
27 100 **HIV serology** will be done on blood samples using rapid tests and according to prevailing national
28
29 101 algorithms. The current algorithm is shown in Appendix 2. This will be done at baseline.

30
31 102 **Pregnancy testing** will be done using urine samples and standard operating procedures for
32
33 103 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline and
34
35 104 before immunisation on each immunisation day.

36
37 105 **Full blood counts** will be conducted using a haematology analyser. Mild, moderate and severe
38
39 106 anaemia will be defined according to WHO guidelines, by age.¹⁴ This will be done at baseline (to test
40
41 107 for anaemia as part of the eligibility assessment), and pre-immunisation as part of the assessment of
42
43 108 immunological profile.

44
45 109 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
46
47 110 care.

48
49 111 Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised
50
51 112 intervention (since the intervention might be beneficial in management of anaemia). They will be
52
53 113 treated for anaemia and for any underlying cause identified.

54 114 ***Operational considerations***

55 115 *Programme governance*

56
57 116 A Programme Steering Committee will be set up to guide progress across all projects. This will
58
59 117 comprise the following:
60

- 1
2
3 118 • An independent chair
4
5 119 • Representatives from the Ministry of Health programmes for immunisation and for vector
6 borne disease control
7 120
8 121 • Representatives of district authorities (Mukono and Jinja districts)
9
10 122 • Community representatives
11
12 123 • Principal investigator and co-investigators
13
14 124 • Project leader and post-doctoral immunologist
15
16 125 • Trial statistician
17
18 126 • Laboratory manager
19
20 127 • Medical Research Council observer

21 128 *Informed consent*

22
23 129 Both written informed assent from the participants and written informed consent from a parent or
24 guardian will be required for participation, although these may not necessarily be obtained at the
25 130 same time. Information will be provided in both English and the appropriate local language. For
26 131 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
27 132 read the information sheet and translate the information to the participant or parent/guardian will
28 133 be used. Informed consent by emancipated or mature minors will be obtained using designated
29 134 consent form for these kinds of participants.
30
31
32
33
34

35 136 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
36 137 will be explained. The participant will be given the opportunity to ask about details of the trial, and
37 138 will then have time to consider whether or not to participate. If they do decide to participate, they
38 139 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
39 140 them to take away and keep, and one to be stored securely by the research team. Separate
40
41
42 141 information and consent forms will be provided for consent for storage of samples for future studies
43 142 and for anonymous sharing of data from this study. For the EMaBS cohort genetic data are already
44 143 available based on previous approval; the information sheet will explain that these data may be used
45 144 in analyses related to this protocol.
46
47
48
49

50
51 145 *Screening and Eligibility Assessment*

52
53 146 Once the informed consent process has been completed, and consent (and assent) given, a baseline
54 147 medical history (including concomitant medication) will be collected. Vital signs will be checked and
55 148 a physical examination will be performed. Inclusion and exclusion criteria will be checked.
56
57
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2
3 149 Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a
4
5 150 trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be
6
7 151 obtained, for tests as specified in the schedule of procedures (Appendices A-C). These tests are to
8
9 152 exclude the major, immunomodulating co-infection, HIV, and conditions that might impact safety
10
11 153 (anaemia, pregnancy).

12 154 *Enrolment*

13
14 155 Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria
15
16 156 and meet none of the exclusion criteria will be enrolled into the trial. On the enrolment day (which
17
18 157 may be the same as the screening day in some cases) eligibility will be checked and participants will
19
20 158 be enrolled sequentially to the next randomisation number. They will then be given BCG vaccine or
21
22 159 not, according to their allocation.

23 160 *Discontinuation / withdrawal criteria*

24
25 161 In accordance with the principles of the current revision of the Declaration of Helsinki and any other
26
27 162 applicable regulations, a participant has the right to withdraw from the study at any time and for any
28
29 163 reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the
30
31 164 participant at any time in the interests of the participant's health and well-being. In addition, the
32
33 165 participant may withdraw/be withdrawn for any of the following reasons:

- 34 166 • Ineligibility (either arising during the study or retrospectively, having been overlooked at
35 167 screening)
- 36 167 • Administrative decision by the Investigator
- 37 168 • Significant protocol deviation
- 38 168 • Participant non-compliance with study requirements
- 39 169 • Participant non-compliance with study requirements
- 40 170 • Participant non-compliance with study requirements
- 41 170 • Participant non-compliance with study requirements
- 42 171 • An adverse event which requires discontinuation of the study involvement or results in
43 171 inability to continue to comply with study procedures.
- 44 172
- 45 172

46
47 173 Any participant who becomes pregnant during the trial will be followed up until the end of the
48
49 174 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
50
51 175 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
52
53 176 will only be given further treatment if clinically indicated. The babies will also be followed up and
54
55 177 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
56
57 178 participant.

1
2
3 179 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
4
5 180 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
6
7 181 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

8
9 182 If a participant withdraws from the study samples collected before their withdrawal from the trial
10
11 183 will be used/ stored unless the participant specifically requests otherwise.

12
13 184 *Trial discontinuation*

14
15 185 The Trial will be discontinued in the event of new scientific information that renders continuation
16
17 186 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

18
19 187 *End of study definition*

20
21 188 The trial will be completed when the last participant enrolled into the trial has completed their final
22
23 189 follow up visit.

24
25 190 *Safety assessments and oversight*

26
27 191 No new investigational drug or product will be used in the proposed trial. However, standard
28
29 192 approaches for monitoring safety and reporting of serious adverse events will be followed.

30
31 193 *Monitoring*

32
33 194 The trial will be monitored by both internal and external monitors according to a pre-defined
34
35 195 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
36
37 196 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
38
39 197 and to Good Clinical Research Practice procedures.

40
41 198 ***Procedures to be followed in the event of abnormal findings***

42
43 199 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
44
45 200 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
46
47 201 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
48
49 202 and appropriate medical care arranged as appropriate and with the permission of the participant.
50
51 203 Specific details regarding findings, discussion with participants and resulting actions will be recorded
52
53 204 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
54
55 205 a participant from the trial will be at the discretion of the Investigator.
56
57
58
59
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1
2
3 206 ***Data and Safety Monitoring Board (DSMB)***
4

5 207 A data and safety monitoring board (DSMB) will be appointed to provide real-time safety oversight.
6

7 208 The DSMB will be notified within 7 days of the Investigators' being aware of the occurrence of SAEs.
8

9 209 The DSMB may recommend the Investigators to place the trial on hold if deemed necessary
10

11 210 following an intervention-related SAE. The DSMB will be chaired by a clinician experienced in clinical
12

13 211 trials. There will be a minimum of two other appropriately qualified committee members. In the case
14

15 212 of events related to a blinded intervention, the DSMB can request unblinding. Membership will
16

17 213 include a statistician, and at least one Ugandan member. All correspondence between Investigators
18

19 214 and the DSMB will be conveyed by the Principal Investigator to the trial Sponsor. The Chair of the
20

21 215 DSMB will be contacted for advice and independent review by the Investigator or trial Sponsor in the
22

23 216 following situations:

24 217

- The occurrence of any SAE

25 218

- Any other situation where the Investigator or trial Sponsor feels independent advice or

26 219 review is important.
27

28
29 220 ***Ethical and regulatory considerations***

30
31 221 *Information regarding risks and benefits to the participant*

32
33 222 Participants in this programme will be adolescents and therefore a vulnerable human population.
34

35 223 Care will be taken to provide adequate, age and education-status appropriate information and to
36

37 224 ensure that it is understood; and to emphasise that participation is voluntary. Participants will be
38

39 225 enrolled only when they have given their own assent and when consent has been given by the
40

41 226 parent or guardian.
42

43 227 No major risks to the participants are anticipated since all the treatments and vaccines to be given
44

45 228 are licensed and known to be safe. The main risk to participants will be time lost from school work:
46

47 229 we will work with parents to minimise disruption to studies.
48

49 230 Participants will suffer the discomfort and inconvenience of providing blood samples (and stool and
50

51 231 urine samples). Occasionally people faint when a vaccine is given or when blood is drawn.
52

53 232 Individuals will be comfortably seated during these procedures and the research team will be trained
54

55 233 to manage such events.
56

57 234 The immunisations to be given have recognised side effects which are usually mild and resolve
58

59 235 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
60

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

1
2
3 238 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
4
5 239 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).¹⁵ Individuals with a history of a
6
7 240 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
8
9 241 proteins, will be excluded from the studies. The research team will be trained and prepared to
10
11 242 manage severe allergic reactions.

12 243 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
13
14 244 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
15
16 245 mortality for this severe, life-threatening adverse effect is reported as about 50%.¹⁵

17 246 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
18
19 247 starting as a small papule at the injection site which may become ulcerated and then heal over a
20
21 248 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
22
23 249 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
24
25 250 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
26
27 251 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
28
29 252 occurs in immunocompromised people: HIV positive people will be excluded from these studies.¹⁶
30
31 253 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
32
33 254 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.¹⁷
34
35 255 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
36
37 256 of neutralising antibody titres (which are the desired protective outcome).^{4 17}

38 257 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
39
40 258 and (rarely) rash.¹⁵

41 259 **Benefits**

42 260 All the vaccines to be given are licensed and regarded as safe. In general, the vaccines and
43
44 261 treatments are expected to provide protection against infectious diseases. Participants and their
45
46 262 families, and communities are expected to benefit from improved understanding of vaccines.

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For peer review only

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN10482904
Funding	#4 Sources and types of financial, material, and other support	15

1	Roles and	#5a	Names, affiliations, and roles of protocol	15
2	responsibilities:		contributors	
3	contributorship			
4				
5				
6	Roles and	#5b	Name and contact information for the trial	Information available at
7	responsibilities:		sponsor	ISRCTN10482904
8	sponsor contact			
9	information			
10				
11				
12				
13	Roles and	#5c	Role of study sponsor and funders, if any, in	16
14	responsibilities:		study design; collection, management, analysis,	
15	sponsor and funder		and interpretation of data; writing of the report;	
16			and the decision to submit the report for	
17			publication, including whether they will have	
18			ultimate authority over any of these activities	
19				
20				
21				
22				
23	Roles and	#5d	Composition, roles, and responsibilities of the	Supplementary
24	responsibilities:		coordinating centre, steering committee, endpoint	information – Pg. 6 and 10
25	committees		adjudication committee, data management team,	
26			and other individuals or groups overseeing the	
27			trial, if applicable (see Item 21a for data	
28			monitoring committee)	
29				
30				
31				
32				
33	Introduction			
34				
35	Background and	#6a	Description of research question and justification	4 and 5
36	rationale		for undertaking the trial, including summary of	
37			relevant studies (published and unpublished)	
38			examining benefits and harms for each	
39			intervention	
40				
41				
42				
43	Background and	#6b	Explanation for choice of comparators	7
44	rationale: choice of			
45	comparators			
46				
47				
48	Objectives	#7	Specific objectives or hypotheses	6
49				
50				
51	Trial design	#8	Description of trial design including type of trial	7
52			(eg, parallel group, crossover, factorial, single	
53			group), allocation ratio, and framework (eg,	
54			superiority, equivalence, non-inferiority,	
55			exploratory)	
56				
57				
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60				

Methods:**Participants,
interventions, and
outcomes**

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 and 7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Supplementary information
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Supplementary information
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a; participants are not expected to be receiving any concomitant care and interventions during the study
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10

1	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Supplementary information; Table S1, pg 2
2				
3				
4				
5				
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8				
9	Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
10				
11				
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17	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	12
18				
19				
20				
21	Methods:			
22	Assignment of			
23	interventions (for			
24	controlled trials)			
25				
26				
27				
28	Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
29				
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41	Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
42				
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49	Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
50				
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54	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care	8
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providers, outcome assessors, data analysts), and how

Blinding (masking): [#17b](#) If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial

Supplementary information – Pg. 10

Methods: Data collection, management, and analysis

Data collection plan [#18a](#) Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol 12

Data collection plan: [#18b](#) Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols 12

Data management [#19](#) Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol 12.

These will also be detailed in a statistical analysis plan that will be uploaded to the online trial registration.

Statistics: outcomes [#20a](#) Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol 12.

These will also be detailed in the statistical analysis plan that will be uploaded to the online trial registration.

1	Statistics: additional	#20b	Methods for any additional analyses (eg,	12.
2	analyses		subgroup and adjusted analyses)	
3				
4				These will also be detailed
5				in the statistical analysis
6				plan that will be uploaded
7				to the online trial
8				registration.
9				
10				
11	Statistics: analysis	#20c	Definition of analysis population relating to	These will be detailed in
12	population and		protocol non-adherence (eg, as randomised	the statistical analysis plan
13	missing data		analysis), and any statistical methods to handle	that will be uploaded to
14			missing data (eg, multiple imputation)	the online trial
15				registration.
16				
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19				
20	Methods:			
21	Monitoring			
22				
23				
24	Data monitoring:	#21a	Composition of data monitoring committee	Supplementary
25	formal committee		(DMC); summary of its role and reporting	information – Pg. 10
26			structure; statement of whether it is independent	
27			from the sponsor and competing interests; and	
28			reference to where further details about its charter	
29			can be found, if not in the protocol. Alternatively,	
30			an explanation of why a DMC is not needed	
31				
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34				
35	Data monitoring:	#21b	Description of any interim analyses and stopping	Supplementary
36	interim analysis		guidelines, including who will have access to	information – Pg. 9
37			these interim results and make the final decision	
38			to terminate the trial	
39				
40				
41				
42	Harms	#22	Plans for collecting, assessing, reporting, and	Supplementary
43			managing solicited and spontaneously reported	information – Pg. 10
44			adverse events and other unintended effects of	
45			trial interventions or trial conduct	
46				
47				
48				
49	Auditing	#23	Frequency and procedures for auditing trial	Supplementary
50			conduct, if any, and whether the process will be	information – Pg. 9
51			independent from investigators and the sponsor	
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Ethics and dissemination

1	Research ethics	#24	Plans for seeking research ethics committee /	11
2	approval		institutional review board (REC / IRB) approval	
3				
4	Protocol	#25	Plans for communicating important protocol	11
5	amendments		modifications (eg, changes to eligibility criteria,	
6			outcomes, analyses) to relevant parties (eg,	
7			investigators, REC / IRBs, trial participants, trial	
8			registries, journals, regulators)	
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12				
13	Consent or assent	#26a	Who will obtain informed consent or assent from	12 and Supplementary
14			potential trial participants or authorised	information – Pg. 7
15			surrogates, and how (see Item 32)	
16				
17				
18	Consent or assent:	#26b	Additional consent provisions for collection and	Supplementary
19	ancillary studies		use of participant data and biological specimens	information – Pg. 7
20			in ancillary studies, if applicable	
21				
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24	Confidentiality	#27	How personal information about potential and	12
25			enrolled participants will be collected, shared, and	
26			maintained in order to protect confidentiality	
27			before, during, and after the trial	
28				
29				
30	Declaration of	#28	Financial and other competing interests for	15
31	interests		principal investigators for the overall trial and	
32			each study site	
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36	Data access	#29	Statement of who will have access to the final	n/a
37			trial dataset, and disclosure of contractual	
38			agreements that limit such access for investigators	
39				
40				
41	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial	Supplementary
42	trial care		care, and for compensation to those who suffer	information – Pg. 10
43			harm from trial participation	
44				
45				
46	Dissemination	#31a	Plans for investigators and sponsor to	2, 12
47	policy: trial results		communicate trial results to participants,	
48			healthcare professionals, the public, and other	
49			relevant groups (eg, via publication, reporting in	
50			results databases, or other data sharing	
51			arrangements), including any publication	
52			restrictions	
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1	Dissemination	#31b	Authorship eligibility guidelines and any intended	n/a
2	policy: authorship		use of professional writers	
3				
4	Dissemination	#31c	Plans, if any, for granting public access to the full	12
5	policy: reproducible		protocol, participant-level dataset, and statistical	
6	research		code	
7				
8				
9				
10	Appendices			
11				
12	Informed consent	#32	Model consent form and other related	n/a
13	materials		documentation given to participants and	
14			authorised surrogates	
15				
16				
17	Biological	#33	Plans for collection, laboratory evaluation, and	n/a
18	specimens		storage of biological specimens for genetic or	
19			molecular analysis in the current trial and for	
20			future use in ancillary studies, if applicable	
21				
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23				

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BMJ Open

The impact of Bacillus Calmette-Guérin 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

Journal:	<i>BMJ Open</i>
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3 1 **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
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3 **20 Abstract**

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5 **21 Introduction**

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There is evidence that Bacillus Calmette–Guérin (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.

26 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 (ages 13 to 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 HPV prime at week 4, HPV boost and Tetanus/diphtheria (Td) boost at week 28. Primary outcomes are anti-YF-17D neutralising antibody titres, *Salmonella typhi* lipopolysaccharide (LPS)-specific IgG concentration, IgG specific for L1-proteins of HPV-16/18 and tetanus and diphtheria toxoid-specific IgG concentration, all assessed at four 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 vs 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.

41 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.

45 Trial registration

Current Controlled Trials identifier: ISRCTN10482904

48 Article summary

49 Strengths and limitations of this study

- 1
2
3 50 • This will be the first well-powered trial to investigate effects of BCG revaccination on
4 responses to unrelated vaccines in adolescents.
5 51
6 52 • Effects on both live-attenuated and inert vaccines will be studied.
7
8 53 • Our robust immunoepidemiological design and nested immunological studies will address
9 specific hypotheses regarding pathways of effects of BCG immunisation on unrelated vaccine
10 54 responses.
11 55
12 56 • One limitation is that interaction between the three vaccines administered together one
13 57 month after BCG immunisation may mask the true effect of BCG revaccination on individual
14 vaccine responses.
15 58
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21 60 **Word count**

22 61 3005

23
24 62 **Keywords**

25 63 Vaccine; BCG; Immunization; Uganda
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64 Introduction

65 There is increasing evidence that *Bacillus Calmette–Guérin* (BCG) immunisation has non-specific,
66 protective effects relating to infections other than tuberculosis.¹⁻⁴ Experimental studies using BCG
67 suggest that effects on the innate immune response are an important component of this
68 phenomenon: BCG immunisation induces lasting epigenetic modification of innate immune cells,
69 including monocytes, macrophages and natural killer cells.⁵⁻⁸ This process, by which the innate
70 immune system develops a form of memory, has been called “trained innate immunity”.⁹ Evidence is
71 accumulating that a range of stimuli including bacterial products (particularly *Salmonella typhi*
72 lipopolysaccharide (LPS)), and infections including malaria and hepatitis B,¹⁰ may induce trained
73 innate immunity; that the profile into which cells are trained varies with the dose and characteristics
74 of the stimulus; and that effects may be induced prenatally (on exposure to maternal infections) as
75 well as later in life.⁹

76 It is plausible that variation in the intensity and spectrum of experience of previous infections, and
77 hence the epigenetic programming and consequent functional profiles of innate immune cells,
78 contributes to the many differences in immunological activity observed between geographically and
79 environmentally distinct settings, and hence to differences in vaccine response. If this hypothesis is
80 correct, BCG immunisation can act as a model for the effects of prior infection, and may also be a
81 tool for inducing enhanced benefits for other vaccines. Vaccine-specific responses can also act as a
82 model for responses to infection. This is especially relevant given the current interest in the
83 potential benefit of BCG immunisation against COVID-19 disease.^{11 12}

84 In Europe, BCG vaccination two weeks before giving influenza vaccine has been shown to result in
85 enhanced antibody responses to influenza proteins.¹³ BCG immunisation four weeks before giving
86 Yellow Fever (YF 17D) vaccine has also been found to result in reduced replication of the yellow
87 fever vaccine virus; this was not associated with a significant reduction in the desired neutralising
88 antibody response to YF, or in the interferon (IFN)- γ response, but the study size was small and may
89 not have had sufficient power to demonstrate important effects.¹⁴

90 In Uganda, BCG immunisation at birth is recommended.¹⁵ The benefits of BCG immunisation in
91 adolescence for protection against tuberculosis are not known and may differ between settings.¹⁶
92 Whether BCG immunisation in adolescents in Uganda will have non-specific effects on the innate
93 immune response, on subsequent immunisations and (indeed) on general health (given the prior
94 exposure at birth, and the on-going exposure to non-tuberculous mycobacteria and other infections)
95 is not known. In Protocol C of the ‘Population differences in Vaccine responses’ programme

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96 (POPVAC C; Current Controlled Trials identifier: ISRCTN10482904), we plan to address this
97 knowledge gap by randomising adolescent members of the Entebbe Mother and Baby Study
98 (EMaBS) birth cohort¹⁵ in a nested trial of BCG revaccination versus no BCG revaccination prior to
99 immunisation with other vaccines. We summarise the protocol here.

For peer review only

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3 100 **Hypothesis**
4

5 101 The overarching goal of the POPVAC programme is to understand population differences in vaccine
6
7 102 responses in Uganda, in order to identify strategies through which vaccine effectiveness can be
8
9 103 optimised for the low-income, tropical settings where they are especially needed. For this Trial C we
10
11 104 address the concept of trained innate immunity through the hypothesis that BCG immunisation
12
13 105 modifies the response to subsequent unrelated vaccines.

14 106 **Objective**
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16 107 To determine whether BCG revaccination modulates the response to unrelated vaccines among
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18 108 Ugandan adolescents.
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109 **Methods and analysis**

110 ***Setting and participants***

111 SPIRIT reporting guidelines¹⁷ are used. This trial will be a randomised, controlled, open, parallel
112 group trial investigating the effect of BCG revaccination on unrelated vaccine response outcomes.
113 The study will take place in Entebbe municipality, Wakiso District, Uganda and will involve
114 participants in the EMaBS birth cohort.¹⁵ In EMaBS, a cohort of 2500 pregnant women were
115 recruited between 2003 and 2005 for a trial of anthelmintic treatment during pregnancy and early
116 childhood, investigating effects on childhood vaccine responses and infectious disease incidence.¹⁵
117 We aim to enroll 300 of the EMaBS birth cohort participants, randomising 150 to each intervention
118 arm. All EMaBS participants received BCG at birth; hence current trial participants (in the BCG
119 intervention arm) will undergo revaccination. EMaBS participants are expected to be aged 13 to 17
120 during recruitment to this study. As part of the on-going cohort follow-up, participants will be
121 encouraged to attend the clinic for interim illness events and all serious adverse events, including
122 hospitalisations, will be documented.

123 ***Recruitment criteria***

124 *Inclusion criteria*

- 125 i. A participant in the Entebbe Mother and Baby Study¹⁵
- 126 ii. Written informed consent by parent or guardian
- 127 iii. Written informed assent by participant
- 128 iv. Willing to remain in the study area for the duration of the study
- 129 v. Willing to provide locator information and to be contacted during the course of the trial
- 130 vi. Females agree to avoid pregnancy for the duration of the trial
- 131 vii. Able and willing (in the investigator's opinion) to comply with all the study requirements

132 *Exclusion criteria*

- 133 i. Concurrent enrolment into another clinical trial
- 134 ii. Clinically significant history of immunodeficiency (including HIV), cancer, cardiovascular
135 disease, gastrointestinal disease, liver disease, renal disease, endocrine disorder and
136 neurological illness
- 137 iii. History of serious psychiatric condition or disorder
- 138 iv. Moderate or severe acute illness characterised by any of the following symptoms: fever,
139 impaired consciousness, convulsions, difficulty in breathing, vomiting; or as determined by
140 the attending project clinician.

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2
3 141 v. History of previous immunisation with Yellow Fever (YF), oral typhoid or Human
4 142 Papillomavirus (HPV) vaccine; previous immunisation with BCG or Tetanus and diphtheria
5 143 vaccine (Td) at age ≥ 5 years
6
7
8 144 vi. Concurrent oral or systemic steroid medication or the concurrent use of other
9 145 immunosuppressive agents within 2 months prior to enrolment
10
11 146 vii. History of allergic reaction to immunisation or any allergy likely to be exacerbated by any
12 147 component of the study vaccines including egg or chicken proteins
13
14
15 148 viii. Tendency to develop keloid scars
16
17 149 ix. Positive HIV serology
18 150 x. Positive pregnancy test
19
20 151 xi. Female currently lactating, confirmed pregnancy or intention to become pregnant during
21 152 the trial period
22
23 153 xii. Use of an investigational medicinal product or non-registered drug, live vaccine, or medical
24 154 device other than the study vaccines for 30 days prior to dosing with the study vaccine, or
25 155 planned use during the study period
26
27
28 156 xiii. Administration of immunoglobulins and/or any blood products within the three months
29 157 preceding the planned trial immunisation date
30
31

32 **Interventions**

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34 159 We will randomise participants to receive BCG or not to receive BCG, four weeks prior to
35 160 immunisation with a panel of licensed unrelated vaccines (discussed below). The adolescents in the
36 161 intervention arm will receive a dose of 0.1 ml of BCG-Russia (Serum Institute of India) in the deltoid
37 162 region of the right upper arm.
38
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41 **Randomisation and allocation to treatment arm**

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43 164 An independent statistician will generate the randomisation code using a randomly permuted block
44 165 size. This code will be embedded as a web-based randomisation system in REDCap (Research
45 166 Electronic Data Capture) software.^{18 19} Randomisation to the two trial arms will be done in a 1:1
46 167 ratio. At enrolment, eligibility criteria will be checked and eligible participants will be allocated
47 168 sequentially to the next randomisation number, with the corresponding trial arm designated in
48 169 REDCap. The randomisation code will be kept securely by the trial statistician with a second copy
49 170 held by a data manager or statistician not otherwise involved in the trial at the MRC/UVRI and
50 171 LSHTM Uganda Research Unit.
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3 **172 Blinding**
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5 173 This trial will not be blinded to clinicians or participants since they will not participate in outcome
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7 174 ascertainment and the expected development of a BCG skin reaction makes blinding difficult. It is
8
9 175 unlikely that participants allocated to “no BCG” will seek this privately. Only laboratory personnel
10
11 176 evaluating vaccine response outcomes will be unaware of BCG allocation so outcome ascertainment
12
13 177 will not be biased through lack of blinding.

14 **178 Immunisations**
15

16 179 We anticipate that BCG revaccination may have different effects on live and non-live, oral and
17
18 180 parenteral, priming and boosting vaccines. Activated innate responses may kill live vaccines and
19
20 181 suppress subsequent adaptive responses by this, or other, mechanisms,^{20,21} but bias, or even
21
22 182 enhance, responses to toxoids or proteins;²²⁻²⁴ thus, results from a single-vaccine study would not
23
24 183 be generalisable.

25 184 We therefore propose to study a portfolio of licensed vaccines (live and inert, oral and parental,
26
27 185 priming and boosting) expected to be beneficial (in some cases, already given) to adolescents in
28
29 186 Uganda. Our schedule (**Table 1** and supplementary **Table S1**) will comprise three main immunisation
30
31 187 days (week 0, week 4 and week 28). Additional HPV immunisation will be provided for girls aged 14
32
33 188 years or above, and a second Td boost will be given after completion of the study, to accord with the
34
35 189 national Expanded Programme on Immunisation (EPI) routines but the response to these will not
36
37 190 specifically be addressed. Further rationale for the selection of vaccines is detailed in supplementary
38
39 191 information. Our schedule has been developed in consultation with the EPI programme and is
40
41 192 cognizant of potential interference between vaccines.

42 193

43 **Table 1. Immunisation schedule**

	Immunisation week 0	Immunisation week 4	[Immunisation week 8]	Immunisation week 28	[Immunisation week 52]
Live vaccines	BCG re-vaccination ¹	Yellow fever (YF-17D) Oral typhoid (Ty21a)			
Non-live vaccines		HPV prime	HPV boost for girls aged ≥ 14 years ^{2,3}	HPV boost and Tetanus/ diphtheria (Td) boost	Tetanus/ diphtheria (Td) boost ^{3,4}
1. 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) 2. The National EPI programme recommends three doses of HPV vaccine for older girls 3. These doses will be given to comply with guidelines but outcomes specifically relating to these doses will not be assessed 4. Priming by immunisation in infancy is assumed					

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3 194 **Schedule of immunisation and sampling**
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5 195 The schedule of immunisation and sampling is outlined in **Table S1**. While optimal timings for
6
7 196 outcome measures vary between vaccines, sampling at 8 weeks post BCG and 4 weeks post YF-17D,
8
9 197 Ty21a, HPV and Td is proposed for the primary endpoints, targeting the establishment of memory
10
11 198 responses and approximate peak of antibody responses. A secondary endpoint at one year will
12
13 199 assess waning. All analyses will take baseline measurements into account. Immunisation
14
15 200 postponement criteria are detailed in Supplementary information.

16 201 **Outcomes**

17
18 202 *Primary outcomes*

19
20 203 These will be assessed in all participants.

- 21
22 204 i. **YF-17D**: neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post
23
24 205 YF immunisation.
25
26 206 ii. **Ty21a**: *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G
27
28 207 concentration at four weeks post Ty21a immunisation.
29
30 208 iii. **HPV**: IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming
31
32 209 immunisation.
33
34 210 iv. **Td**: tetanus and diphtheria toxoid-specific IgG concentration at four weeks post Td
35
36 211 immunisation.

37 212 *Secondary outcomes*

38
39 213 These will be assessed in all participants and will further investigate estimates of protective
40
41 214 immunity (for vaccines where these are available) and dynamics of the vaccine responses, as well as
42
43 215 the impact of the interventions on parasite clearance.

- 44 216 i. **Protective immunity**: Proportions with protective neutralising antibody (YF); protective IgG
45
46 217 levels (TT);²⁵ seroconversion rates (Ty21a) at four weeks post the corresponding
47
48 218 immunisation.
49
50 219 ii. **Response waning**: Primary outcome measures (all vaccines) repeated at week 52, and area-
51
52 220 under-the curve (AUC) analyses. Parasitic infection may accelerate,²⁶ and anti-parasitic
53
54 221 interventions delay, waning.
55
56 222 iii. **Priming versus boosting**: Effects on priming versus boosting will be examined for HPV only,
57
58 223 comparing outcomes four weeks after the first, and four weeks after the second vaccine
59
60 224 dose.

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

232 *Additional measurements*

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

236 *Sample size considerations*

237 Based on the literature^{20 27 28} and preliminary data, we anticipate that standard deviations (SDs) of
 238 primary outcome measures will lie between 0.3 and 0.6 log₁₀; and that revaccination with BCG may
 239 increase responses by approximately 0.12-0.14 log₁₀. Based on these assumptions, we aim to enrol
 240 300 EMaBS participants (150 BCG revaccination, 150 no BCG revaccination). Allowing for 10% loss to
 241 follow-up, this will give over 90% power to detect a difference of 0.12log₁₀ in vaccine response
 242 between the pre-BCG immunised and non-pre-immunised groups, at 5% significance level and
 243 assuming vaccine response standard deviation of 0.3log₁₀ (**Table 2**).

Table 2. Power estimates (5% significance level)

Standard deviation (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.

245

246 *Ethics and Dissemination*

247 Ethical approval has been granted from the Research Ethics Committees of the Uganda Virus
 248 Research Institute (reference: GC/127/19/05/682), the London School of Hygiene and Tropical
 249 Medicine (reference: 16034), the Uganda National Council for Science and Technology (reference:
 250 HS 2491) and from the Uganda National Drug Authority (certificate number: CTA0094). Any protocol

1
2
3 251 amendments will be submitted to ethics committees and regulatory bodies for approval before
4
5 252 implementation.

6
7 253 Participants will be adolescents and therefore a vulnerable human population. Care will be taken to
8
9 254 provide adequate, age- and education-status appropriate information and to ensure that it is
10
11 255 understood; and to emphasise that participation is voluntary. Participants will be enrolled only when
12
13 256 they have given their own assent and when consent has been given by the parent or guardian. No
14
15 257 major risks to the participants are anticipated since all the vaccines to be given are licensed and
16
17 258 known to be safe.

18 259 With regard to BCG immunisation or revaccination in adolescence, benefits with respect to
19
20 260 protection against tuberculosis among Ugandan adolescents are unknown and may, at best, be
21
22 261 modest. There may be non-specific benefits. WHO's SAGE committee concluded, in their summary
23
24 262 of October 2017,²⁹ that "BCG revaccination is safe in *Mycobacterium tuberculosis* infected and
25
26 263 uninfected populations. There is a lack of evidence from randomised controlled trials and
27
28 264 retrospective cohort and case-control studies demonstrating the efficacy and effectiveness of BCG
29
30 265 revaccination in adolescents and adults after primary BCG vaccination in infancy for protection
31
32 266 against TB disease. Due to absence of evidence, BCG revaccination is not considered cost-effective.
33
34 267 Further research is warranted to explore whether certain sub-groups of age, geographic or *M.*
35
36 268 *tuberculosis* exposure categories would benefit from BCG revaccination." We hope, through this
37
38 269 work, to contribute to this debate.

39
40 270 Study findings will be published through open access peer-reviewed journals, presentations at local,
41
42 271 national and international conferences and to the local community through community meetings.
43
44 272 Anonymised participant level datasets generated will be available upon request.

45 273 ***Patient and public involvement***

46 274 The EMaBS research team has previously worked with volunteer local council field workers to ensure
47
48 275 regular follow up of participants and these field workers continue to attend participants' meetings
49
50 276 and provide a mechanism by which the communities from which participants are drawn can be
51
52 277 informed about on-going work. As well, prior to the start of this study, we will share our plans with
53
54 278 district health and education officers, and with colleagues at Entebbe Hospital. We will establish an
55
56 279 advisory committee of parents who will help us to ensure that EMaBS cohort members can
57
58 280 participate in the study without undue disruption to their school work. Study findings will be shared
59
60 281 with these stakeholders and with participants.

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3 282 ***Data management and analysis***
4

5 283 Socio-demographic information and clinical and laboratory measurements will be recorded and
6 284 managed using REDCap (Research Electronic Data Capture) tools,^{18 19} with paper-based forms as
7
8 285 back-up. All data will be recorded under a unique study ID number. When paper forms must be
9
10 286 used, data will be double entered in a study-specific database, with standard checks for
11
12 287 discrepancies. All data for analysis will be anonymised and stored on a secure and password-
13
14 288 protected server, with access limited to essential research personnel.

15
16 289 The effect of BCG versus no BCG revaccination on the outcomes will be analysed, including sub
17
18 290 group analysis by sex. The analysis will test whether BCG pre-immunisation alters the response to
19
20 291 live or inert vaccines given four weeks later, including effects on vaccine replication, immune
21
22 292 response profile, priming, boosting and waning. It will indicate whether including BCG as a
23
24 293 component of school-based immunisation schedules is likely to have non-specific benefits for
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26 294 Ugandan adolescents.
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3 **295 Discussion**
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5 296 It is increasingly clear that several live vaccines, including BCG, measles vaccine and Vaccinia
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7 297 (smallpox) vaccine, have non-specific, beneficial, effects including reduced mortality (not related to
8
9 298 the infectious disease that they were designed to target).^{1,2} The potential effects of BCG on
10
11 299 responses to unrelated vaccines, specifically on live-attenuated ones such as yellow fever and oral
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13 300 typhoid, might model its effects on responses to unrelated infectious agents.

14 301 In contrast, non-specific negative effects have been associated with inactivated vaccines such as
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16 302 diphtheria-tetanus-pertussis (DTP). A high childhood mortality has been observed among girls
17
18 303 vaccinated with DTP.^{30,31} It has been further suggested that reducing time of exposure to DTP as the
19
20 304 most recent vaccination with BCG may reduce this childhood mortality.³⁰

21 305 We hypothesise that BCG immunisation both achieves non-specific benefits, and influences vaccine
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23 306 responses, through mechanisms based on effects on the innate immune system and consequent
24
25 307 immunological profile.

26
27 308 Of note, in this Ugandan birth cohort, all participants were documented to have received BCG at
28
29 309 birth, with the strain of BCG used recorded.¹⁵ This will therefore be the first well-powered study to
30
31 310 investigate effects of BCG revaccination on vaccine responses in adolescents. It will not investigate
32
33 311 the effects of a first dose of BCG in adolescence.

34 312 For this work, all participants will receive BCG-Russia strain, provided by the Serum Institute of India.
35
36 313 While responses to strains vary, this strain is widely available globally, and in use in Uganda. For
37
38 314 comparability, it will be used across the three trials, POPVAC A, B and C. In the context of these
39
40 315 trials it will not be possible to determine whether different strains of BCG would have different
41
42 316 effects on other vaccines.

43 317 This study will determine whether BCG immunisation alters the response to live or inert vaccines
44
45 318 given four weeks later, including effects on vaccine replication, immune response profile, priming,
46
47 319 boosting and waning among adolescents who received BCG as infants. It will indicate whether
48
49 320 including BCG as a component of school-based immunisation schedules is likely to have non-specific
50
51 321 benefits for Ugandan adolescents and other settings where infant BCG immunisation is common. If
52
53 322 this is correct, BCG immunisation may be used as a tool for inducing enhanced benefits for other
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55 323 vaccines in a wide range of settings.

56 324

57 **325 Study timeline**
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3 326 Applications for ethical approval were submitted in May 2018, with approval received in September
4
5 327 2018 (Uganda Virus Research Institute Research Ethics Committee), May 2019 (National Drug
6
7 328 Authority and Uganda National Council for Science and Technology) and June 2019 (London School
8
9 329 of Hygiene and Tropical Medicine). Collaborator/investigator/trial steering committee meetings
10
11 330 were also held during the initial 12-month planning period. Recruitment is scheduled to commence
12
13 331 in May 2020. Intervention will be up to 12 months, with completion of the project scheduled for
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15 332 April 2022.
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3 333 **Competing interests**
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5 334 Alison Elliott reports a grant from the Medical research Council, UK (POPVAC programme funding).
6

7 335 The rest of the authors declare that they have no conflicts of interest.
8

9 336 **Author contributions**
10

11 337 AME conceived the study. AME, GN, ELW, AN, AW, SC, LZ and MM contributed to study design. LZ,
12

13 338 GO, GK, JS, CO, MN, EN, FA and JT are site clinicians/nurses/clinical laboratory technicians providing
14

15 339 valuable input on clinical considerations of the intervention. MS, SK, FK, RK and MK are field workers
16

17 340 and administrators handling the organisational integration of the intervention. AN, AM, HA and ELW
18

19 341 are involved in organisation of the databases, trial randomization, treatment allocation and drawing
20

21 342 up of analytical plans. LZ, GN, JN, AN, SC, ELW and AME drafted the manuscript. All authors reviewed
22

23 343 the manuscript, contributed to it and approved the final version.
24

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27

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29

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31

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33

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35

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37

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42

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44

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46

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48

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50

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52

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54

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56

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58

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60

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3 363 The study sponsor (London School of Hygiene and Tropical Medicine) and funders had no role in
4
5 364 study design; collection, management, analysis, and interpretation of data; writing of the protocol;
6
7 365 and the decision to submit the protocol for publication.
8
9 366

10
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3 1 **SUPPLEMENTARY INFORMATION**
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5 2

6
7 3 **The impact of Bacillus Calmette-Guérin revaccination on the response to unrelated vaccines in a**
8 **Ugandan adolescent birth cohort: randomised controlled trial protocol C for the ‘POPulation**
9 **differences in VACCine responses’ (POPVAC) programme**
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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 1. Screening and enrolment into Project C will take place shortly before enrolment, sometimes on the same day</p>							

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- 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.drgreene.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

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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.

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3 88 **Prior exposure to schistosomiasis** will be evaluated by ELISA for IgG to schistosome egg antigen
4
5 89 using stored blood samples collected at baseline.

6
7 90 **The presence of other helminth infections** will be determined retrospectively using stool PCR of
8
9 91 samples collected at baseline and at weeks 28 and 52.¹³ In accordance with national guidelines, all
10
11 92 participants will be treated with albendazole or mebendazole after collection of samples for primary
12
13 93 endpoints at week 8 and 28, and after collection of samples for secondary endpoints at week 52.

14
15 94 **Current malaria infection status and intensity** will be assessed retrospectively by PCR on stored
16
17 95 samples collected on immunisation days and at week 52.

18
19 96 **Malarial fever:** Individuals presenting with fever will be investigated using rapid diagnostic tests for
20
21 97 malaria and treated based on the results and according to prevailing national guidelines.

22
23 98 **Prior malaria exposure** will be evaluated by ELISA for IgG to malaria antigen using stored samples
24
25 99 collected at baseline.

26 100 **HIV serology** will be done on blood samples using rapid tests and according to prevailing national
27
28 101 algorithms. The current algorithm is shown in Appendix 2. This will be done at baseline.

29
30 102 **Pregnancy testing** will be done using urine samples and standard operating procedures for
31
32 103 assessment of urine β -human chorionic gonadotropin (β hCG). This will be done at baseline and
33
34 104 before immunisation on each immunisation day.

35
36 105 **Full blood counts** will be conducted using a haematology analyser. Mild, moderate and severe
37
38 106 anaemia will be defined according to WHO guidelines, by age.¹⁴ This will be done at baseline (to test
39
40 107 for anaemia as part of the eligibility assessment), and pre-immunisation as part of the assessment of
41
42 108 immunological profile.

43
44 109 Individuals found to be HIV positive or pregnant will be referred to appropriate providers for further
45
46 110 care.

47
48 111 Individuals with severe anaemia (haemoglobin <82g/L) will be excluded from the randomised
49
50 112 intervention (since the intervention might be beneficial in management of anaemia). They will be
51
52 113 treated for anaemia and for any underlying cause identified.

53 114 ***Operational considerations***

54 115 *Programme governance*

55
56 116 A Programme Steering Committee will be set up to guide progress across all projects. This will
57
58 117 comprise the following:
59
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3 118 • An independent chair
4
5 119 • Representatives from the Ministry of Health programmes for immunisation and for vector
6 borne disease control
7 120
8 121 • Representatives of district authorities (Mukono and Jinja districts)
9
10 122 • Community representatives
11
12 123 • Principal investigator and co-investigators
13
14 124 • Project leader and post-doctoral immunologist
15
16 125 • Trial statistician
17
18 126 • Laboratory manager
19
20 127 • Medical Research Council observer

21 128 *Informed consent*

22
23 129 Both written informed assent from the participants and written informed consent from a parent or
24 guardian will be required for participation, although these may not necessarily be obtained at the
25 130 same time. Information will be provided in both English and the appropriate local language. For
26 131 individuals who cannot speak the languages used, or who cannot read or write, a witness who can
27 132 read the information sheet and translate the information to the participant or parent/guardian will
28 133 be used. Informed consent by emancipated or mature minors will be obtained using designated
29 134 consent form for these kinds of participants.
30
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35 136 The aims of the study, all tests, treatments and immunisations to be carried out and potential risks
36 137 will be explained. The participant will be given the opportunity to ask about details of the trial, and
37 138 will then have time to consider whether or not to participate. If they do decide to participate, they
38 139 and their parent/guardian will sign and date two copies of the assent and consent forms, one for
39 140 them to take away and keep, and one to be stored securely by the research team. Separate
40 141 information and consent forms will be provided for consent for storage of samples for future studies
41 142 and for anonymous sharing of data from this study. For the EMaBS cohort genetic data are already
42 143 available based on previous approval; the information sheet will explain that these data may be used
43 144 in analyses related to this protocol.
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51 145 *Screening and Eligibility Assessment*

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53 146 Once the informed consent process has been completed, and consent (and assent) given, a baseline
54 147 medical history (including concomitant medication) will be collected. Vital signs will be checked and
55 148 a physical examination will be performed. Inclusion and exclusion criteria will be checked.
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3 149 Participants will undergo pre- and post-test counselling for HIV and (for girls) pregnancy testing by a
4
5 150 trained and experienced nurse- or clinician-counsellor. Blood, urine and stool samples will be
6
7 151 obtained, for tests as specified in the schedule of procedures (Appendices A-C). These tests are to
8
9 152 exclude the major, immunomodulating co-infection, HIV, and conditions that might impact safety
10
11 153 (anaemia, pregnancy).

12 154 *Enrolment*

13
14 155 Participants who consent/assent, complete the screening processes, satisfy all the inclusion criteria
15
16 156 and meet none of the exclusion criteria will be enrolled into the trial. On the enrolment day (which
17
18 157 may be the same as the screening day in some cases) eligibility will be checked and participants will
19
20 158 be enrolled sequentially to the next randomisation number. They will then be given BCG vaccine or
21
22 159 not, according to their allocation.

23 160 *Discontinuation / withdrawal criteria*

24
25 161 In accordance with the principles of the current revision of the Declaration of Helsinki and any other
26
27 162 applicable regulations, a participant has the right to withdraw from the study at any time and for any
28
29 163 reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the
30
31 164 participant at any time in the interests of the participant's health and well-being. In addition, the
32
33 165 participant may withdraw/be withdrawn for any of the following reasons:

- 34 166 • Ineligibility (either arising during the study or retrospectively, having been overlooked at
35 167 screening)
- 36 168 • Administrative decision by the Investigator
- 37 169 • Significant protocol deviation
- 38 170 • Participant non-compliance with study requirements
- 39 171 • An adverse event which requires discontinuation of the study involvement or results in
40 172 inability to continue to comply with study procedures.

41
42
43 173 Any participant who becomes pregnant during the trial will be followed up until the end of the
44
45 174 pregnancy but no further immunisations will be given unless indicated during pregnancy (as is the
46
47 175 case for tetanus toxoid). The trial allocation for this participant will be unblinded and the participant
48
49 176 will only be given further treatment if clinically indicated. The babies will also be followed up and
50
51 177 examined for any adverse effects. We will not routinely perform venepuncture in a pregnant
52
53 178 participant.
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3 179 The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an
4
5 180 AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the
6
7 181 participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

8
9 182 If a participant withdraws from the study samples collected before their withdrawal from the trial
10
11 183 will be used/ stored unless the participant specifically requests otherwise.

12 184 *Trial discontinuation*

13
14 185 The Trial will be discontinued in the event of new scientific information that renders continuation
15
16 186 futile or unethical, or for any other reason, at the discretion of the Programme Steering Committee.

17 187 *End of study definition*

18
19 188 The trial will be completed when the last participant enrolled into the trial has completed their final
20
21 189 follow up visit.

22 190 *Safety assessments and oversight*

23
24 191 No new investigational drug or product will be used in the proposed trial. However, standard
25
26 192 approaches for monitoring safety and reporting of serious adverse events will be followed.

27 193 *Monitoring*

28
29 194 The trial will be monitored by both internal and external monitors according to a pre-defined
30
31 195 monitoring plan which will include a site initiation visit, monitoring visits at least annually, and a
32
33 196 close-out visit. The monitors will assess patient safety, data integrity, and adherence to the protocol
34
35 197 and to Good Clinical Research Practice procedures.

36 198 ***Procedures to be followed in the event of abnormal findings***

37
38 199 Abnormal clinical findings from medical history, examination or blood tests will be assessed as to
39
40 200 their clinical significance throughout the trials. If an abnormal test result is deemed clinically
41
42 201 significant, it may be repeated. If a test remains clinically significant, the participant will be informed
43
44 202 and appropriate medical care arranged as appropriate and with the permission of the participant.
45
46 203 Specific details regarding findings, discussion with participants and resulting actions will be recorded
47
48 204 in the clinical records. Decisions to exclude the participant from enrolling in the trial or to withdraw
49
50 205 a participant from the trial will be at the discretion of the Investigator.
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3 206 ***Data and Safety Monitoring Board (DSMB)***
4

5 207 A data and safety monitoring board (DSMB) will be appointed to provide real-time safety oversight.
6

7 208 The DSMB will be notified within 7 days of the Investigators' being aware of the occurrence of SAEs.
8

9 209 The DSMB may recommend the Investigators to place the trial on hold if deemed necessary
10

11 210 following an intervention-related SAE. The DSMB will be chaired by a clinician experienced in clinical
12

13 211 trials. There will be a minimum of two other appropriately qualified committee members. In the case
14

15 212 of events related to a blinded intervention, the DSMB can request unblinding. Membership will
16

17 213 include a statistician, and at least one Ugandan member. All correspondence between Investigators
18

19 214 and the DSMB will be conveyed by the Principal Investigator to the trial Sponsor. The Chair of the
20

21 215 DSMB will be contacted for advice and independent review by the Investigator or trial Sponsor in the
22

23 216 following situations:

24 217

- The occurrence of any SAE

25 218

- Any other situation where the Investigator or trial Sponsor feels independent advice or

26 219 review is important.
27

28
29 220 ***Ethical and regulatory considerations***

30
31 221 *Information regarding risks and benefits to the participant*

32
33 222 Participants in this programme will be adolescents and therefore a vulnerable human population.
34

35 223 Care will be taken to provide adequate, age and education-status appropriate information and to
36

37 224 ensure that it is understood; and to emphasise that participation is voluntary. Participants will be
38

39 225 enrolled only when they have given their own assent and when consent has been given by the
40

41 226 parent or guardian.
42

43 227 No major risks to the participants are anticipated since all the treatments and vaccines to be given
44

45 228 are licensed and known to be safe. The main risk to participants will be time lost from school work:
46

47 229 we will work with parents to minimise disruption to studies.
48

49 230 Participants will suffer the discomfort and inconvenience of providing blood samples (and stool and
50

51 231 urine samples). Occasionally people faint when a vaccine is given or when blood is drawn.
52

53 232 Individuals will be comfortably seated during these procedures and the research team will be trained
54

55 233 to manage such events.
56

57 234 The immunisations to be given have recognised side effects which are usually mild and resolve
58

59 235 spontaneously in a few days to one week. Parenteral vaccines are likely to result in pain and
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236 swelling at the site of injection and mild fever; very occasionally pain and swelling can be severe and
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associated with difficulty in moving the shoulder. Sometimes headache and tiredness occurs. Rarely

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3 238 a vaccine may cause a severe allergic reaction. For most vaccines this is estimated at less than one
4
5 239 in a million doses (but 1 in 55,000 for Yellow Fever vaccine).¹⁵ Individuals with a history of a
6
7 240 possible allergic reaction to drugs or vaccines, or to vaccine components including eggs or chicken
8
9 241 proteins, will be excluded from the studies. The research team will be trained and prepared to
10
11 242 manage severe allergic reactions.

12 243 Adverse reactions to Yellow Fever vaccine include severe nervous system reaction (about 1 person in
13
14 244 125,000) and severe, life-threatening illness with organ failure (about 1 person in 250,000). The
15
16 245 mortality for this severe, life-threatening adverse effect is reported as about 50%.¹⁵

17 246 BCG immunisation is likely to induce a scar in many cases. This may develop over several weeks,
18
19 247 starting as a small papule at the injection site which may become ulcerated and then heal over a
20
21 248 period of 2 to 5 months; and lymphadenopathy may develop. Occasionally a more severe local
22
23 249 reaction occurs (estimated at 1 per 1,000-10,000 doses): for example, an abscess develops and scars
24
25 250 may develop into keloids. Rarely BCG can cause disseminated disease (1 per 230,000 to 640,000
26
27 251 doses), or disease in sites remote from the immunisation site. Disseminated BCG disease usually
28
29 252 occurs in immunocompromised people: HIV positive people will be excluded from these studies.¹⁶
30
31 253 BCG "pre-immunisation" may interfere with the response to the subsequent live vaccines; indeed
32
33 254 our hypothesis, and published results, suggest that it may suppress replication of YF 17D vaccine.¹⁷
34
35 255 However, this reduced replication has not been shown to correlate with, or result in, reduced levels
36
37 256 of neutralising antibody titres (which are the desired protective outcome).^{4 17}

38 257 Oral typhoid vaccine (Ty21a) may occasionally be associated with stomach pain, nausea, vomiting
39
40 258 and (rarely) rash.¹⁵

41 259 **Benefits**

42 260 All the vaccines to be given are licensed and regarded as safe. In general, the vaccines and
43
44 261 treatments are expected to provide protection against infectious diseases. Participants and their
45
46 262 families, and communities are expected to benefit from improved understanding of vaccines.

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304

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207

	Reporting Item	Page Number
Administrative information		
Title	#1 Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3 Date and version identifier	Information available at ISRCTN10482904
Funding	#4 Sources and types of financial, material, and other support	16

1	Roles and	#5a	Names, affiliations, and roles of protocol	16
2	responsibilities:		contributors	
3	contributorship			
4				
5				
6	Roles and	#5b	Name and contact information for the trial	Information available at
7	responsibilities:		sponsor	ISRCTN10482904
8	sponsor contact			
9	information			
10				
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12				
13	Roles and	#5c	Role of study sponsor and funders, if any, in	17
14	responsibilities:		study design; collection, management, analysis,	
15	sponsor and funder		and interpretation of data; writing of the report;	
16			and the decision to submit the report for	
17			publication, including whether they will have	
18			ultimate authority over any of these activities	
19				
20				
21				
22				
23	Roles and	#5d	Composition, roles, and responsibilities of the	Supplementary
24	responsibilities:		coordinating centre, steering committee, endpoint	information – Pg. 6 and 10
25	committees		adjudication committee, data management team,	
26			and other individuals or groups overseeing the	
27			trial, if applicable (see Item 21a for data	
28			monitoring committee)	
29				
30				
31				
32				
33	Introduction			
34				
35	Background and	#6a	Description of research question and justification	4 and 5
36	rationale		for undertaking the trial, including summary of	
37			relevant studies (published and unpublished)	
38			examining benefits and harms for each	
39			intervention	
40				
41				
42				
43	Background and	#6b	Explanation for choice of comparators	7
44	rationale: choice of			
45	comparators			
46				
47				
48	Objectives	#7	Specific objectives or hypotheses	6
49				
50				
51	Trial design	#8	Description of trial design including type of trial	7
52			(eg, parallel group, crossover, factorial, single	
53			group), allocation ratio, and framework (eg,	
54			superiority, equivalence, non-inferiority,	
55			exploratory)	
56				
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59				
60				

Methods:**Participants,
interventions, and
outcomes**

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 and 7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Supplementary information
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Supplementary information
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a; participants are not expected to be receiving any concomitant care and interventions during the study
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10

1	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Supplementary information; Table S1, pg 2
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9	Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
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17	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	12
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21	Methods:			
22	Assignment of			
23	interventions (for			
24	controlled trials)			
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28	Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
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41	Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
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49	Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
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54	Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care	8
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providers, outcome assessors, data analysts), and how

Blinding (masking): [#17b](#) If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial

Supplementary information – Pg. 10

Methods: Data collection, management, and analysis

Data collection plan [#18a](#) Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol 13

Data collection plan: [#18b](#) Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols 13

Data management [#19](#) Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol 13.

These will also be detailed in a statistical analysis plan that will be uploaded to the online trial registration.

Statistics: outcomes [#20a](#) Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol 13.

These will also be detailed in the statistical analysis plan that will be uploaded to the online trial registration.

1	Statistics: additional	#20b	Methods for any additional analyses (eg,	13.
2	analyses		subgroup and adjusted analyses)	
3				These will also be detailed
4				in the statistical analysis
5				plan that will be uploaded
6				to the online trial
7				registration.
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11	Statistics: analysis	#20c	Definition of analysis population relating to	These will be detailed in
12	population and		protocol non-adherence (eg, as randomised	the statistical analysis plan
13	missing data		analysis), and any statistical methods to handle	that will be uploaded to
14			missing data (eg, multiple imputation)	the online trial
15				registration.
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20	Methods:			
21	Monitoring			
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24	Data monitoring:	#21a	Composition of data monitoring committee	Supplementary
25	formal committee		(DMC); summary of its role and reporting	information – Pg. 10
26			structure; statement of whether it is independent	
27			from the sponsor and competing interests; and	
28			reference to where further details about its charter	
29			can be found, if not in the protocol. Alternatively,	
30			an explanation of why a DMC is not needed	
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35	Data monitoring:	#21b	Description of any interim analyses and stopping	Supplementary
36	interim analysis		guidelines, including who will have access to	information – Pg. 9
37			these interim results and make the final decision	
38			to terminate the trial	
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42	Harms	#22	Plans for collecting, assessing, reporting, and	Supplementary
43			managing solicited and spontaneously reported	information – Pg. 10
44			adverse events and other unintended effects of	
45			trial interventions or trial conduct	
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49	Auditing	#23	Frequency and procedures for auditing trial	Supplementary
50			conduct, if any, and whether the process will be	information – Pg. 9
51			independent from investigators and the sponsor	
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Ethics and dissemination

1	Research ethics	#24	Plans for seeking research ethics committee /	11
2	approval		institutional review board (REC / IRB) approval	
3				
4	Protocol	#25	Plans for communicating important protocol	11
5	amendments		modifications (eg, changes to eligibility criteria,	
6			outcomes, analyses) to relevant parties (eg,	
7			investigators, REC / IRBs, trial participants, trial	
8			registries, journals, regulators)	
9				
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13	Consent or assent	#26a	Who will obtain informed consent or assent from	12 and Supplementary
14			potential trial participants or authorised	information – Pg. 7
15			surrogates, and how (see Item 32)	
16				
17				
18	Consent or assent:	#26b	Additional consent provisions for collection and	Supplementary
19	ancillary studies		use of participant data and biological specimens	information – Pg. 7
20			in ancillary studies, if applicable	
21				
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24	Confidentiality	#27	How personal information about potential and	12
25			enrolled participants will be collected, shared, and	
26			maintained in order to protect confidentiality	
27			before, during, and after the trial	
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30	Declaration of	#28	Financial and other competing interests for	16
31	interests		principal investigators for the overall trial and	
32			each study site	
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36	Data access	#29	Statement of who will have access to the final	n/a
37			trial dataset, and disclosure of contractual	
38			agreements that limit such access for investigators	
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41	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial	Supplementary
42	trial care		care, and for compensation to those who suffer	information – Pg. 10
43			harm from trial participation	
44				
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46	Dissemination	#31a	Plans for investigators and sponsor to	2, 11
47	policy: trial results		communicate trial results to participants,	
48			healthcare professionals, the public, and other	
49			relevant groups (eg, via publication, reporting in	
50			results databases, or other data sharing	
51			arrangements), including any publication	
52			restrictions	
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1	Dissemination	#31b	Authorship eligibility guidelines and any intended	n/a
2	policy: authorship		use of professional writers	
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4	Dissemination	#31c	Plans, if any, for granting public access to the full	11
5	policy: reproducible		protocol, participant-level dataset, and statistical	
6	research		code	
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10	Appendices			
11				
12	Informed consent	#32	Model consent form and other related	Supplementary File
13	materials		documentation given to participants and	
14			authorised surrogates	
15				
16				
17	Biological	#33	Plans for collection, laboratory evaluation, and	n/a
18	specimens		storage of biological specimens for genetic or	
19			molecular analysis in the current trial and for	
20			future use in ancillary studies, if applicable	
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 26 [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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