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**PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent placental malaria: a first-in-human, randomised, double-blind, placebo-controlled study**

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► **To cite this version:**

Sodiomon B Sirima, Laura Richert, Arnaud Chêne, Amadou T Konate, Cécilia Campion, et al.. PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent placental malaria: a first-in-human, randomised, double-blind, placebo-controlled study. *The Lancet Infectious Diseases*, 2020, 20 (5), pp.585-597. 10.1016/S1473-3099(19)30739-X . hal-03161802v2

**HAL Id: hal-03161802**

**<https://inria.hal.science/hal-03161802v2>**

Submitted on 2 Dec 2020

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1 **PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent**  
2 **placental malaria: a first-in-human, randomised, double-blind, placebo-**  
3 **controlled study**

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88 **Abstract**

89 ***Background:***

90 PRIMVAC is a VAR2CSA-derived placental malaria (PM) vaccine candidate aiming to prevent  
91 serious clinical outcomes of *Plasmodium falciparum* infection during pregnancy. In this phase  
92 Ia/Ib clinical trial, we assessed the safety and immunogenicity of PRIMVAC adjuvanted with  
93 Alhydrogel® or Glucopyranosyl Lipid Adjuvant in stable emulsion (GLA-SE) in non-pregnant  
94 French and Burkinabe women.

95

96 ***Methods:***

97 This first-in-human randomised, double-blind, placebo-controlled, dose escalation trial was  
98 conducted in two staggered phases, a phase Ia in 18-35 years old malaria naïve women in France  
99 and a subsequent phase Ib in malaria exposed nulligravid women in Burkina Faso. Volunteers  
100 were recruited into four sequential cohorts receiving PRIMVAC intra-muscularly at day 0 (D0),  
101 day 28 (D28) and day 56 (D56): two in France receiving 20 µg or 50 µg of PRIMVAC and then  
102 two in Burkina-Faso receiving 50 µg or 100 µg of PRIMVAC. Within each cohort, volunteers  
103 were randomised to two arms (PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE) in  
104 France and three arms (PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE or placebo)  
105 in Burkina Faso. The primary endpoint of the study was the proportion of participants with any  
106 grade 3 or higher adverse reaction to vaccination until day 35. Safety at later time points as well  
107 as humoral and cellular immunogenicity were assessed in secondary endpoints. This trial is  
108 registered with ClinicalTrials.gov, number NCT02658253.

109

110 ***Findings:***

111 Between April 19 2016, and July 13 2017, a total of 68 women (18 in France, 50 in Burkina  
112 Faso) were included. No serious adverse event following immunization (SAEFI) related to the

113 vaccine occurred. PRIMVAC antibody titres increased with each dose and seroconversion was  
114 observed in all the PRIMVAC vaccinated women (N=57). PRIMVAC antibody titres reached  
115 a peak (geometric mean 11843·0 OD 1·0, 95% CI 7559·8-18552·9 with 100 µg dose and GLA-  
116 SE) one week after the third vaccination (Day 63). In comparison to Alhydrogel®, GLA-SE  
117 tended to improve the PRIMVAC antibody response (geometric mean 2163·5 OD 1·0, 95% CI  
118 1315·7-3557·7 with 100 µg dose and Alhydrogel at Day 63). One year after the last vaccination,  
119 20 out of 28 (71·4%) PRIMVAC/Alhydrogel® and 26 out of 28 (92·9%) PRIMVAC/GLA-SE  
120 vaccinated women still had PRIMVAC antibodies, although antibody magnitude was markedly  
121 lower (452·4 OD 1·0, 95% CI 321·8-636·1 with 100 µg dose and GLA-SE). These antibodies  
122 reacted with native homologous VAR2CSA expressed by NF54-CSA infected erythrocytes  
123 (IEs) (fold change from baseline at Day 63 with 100 µg dose and GLA-SE: 10·74, 95% CI  
124 8·36-13·79). Limited cross-recognition, restricted to sera collected from women that have  
125 received the 100 µg PRIMVAC dosage, was observed against heterologous VAR2CSA variants  
126 expressed by FCR3-CSA and 7G8-CSA IEs (fold change from baseline at Day 63 1·49, 95% CI  
127 1·19-1·88 and 1·2, 95% CI 1·08-1·34, respectively). PRIMVAC antibodies also inhibit the  
128 interactions between the homologous VAR2CSA expressed on NF54-CSA IEs and its placental  
129 receptor, chondroitin sulfate A (CSA) (% inhibition from baseline at Day 63 with 100 µg dose  
130 and GLA-SE: 58·3, 95% CI 48·5-68·1).

131

132 ***Interpretation:***

133 PRIMVAC adjuvanted with Alhydrogel® or GLA-SE had an acceptable safety profile, was  
134 immunogenic and induced functional antibodies reacting with the homologous VAR2CSA  
135 variant expressed by NF54-CSA IEs. Limited cross-reactivity against heterologous VAR2CSA  
136 variants was only observed in the higher dosage group. Other schedule of immunization,

137 antigen dosage and combinations with other VAR2CSA-based vaccines are envisaged to  
138 improve the cross-reactivity.

139

140 ***Funding:***

141 Bundesministerium für Bildung und Forschung, through Kreditanstalt für Wiederaufbau (ref:  
142 202060457), Germany; Inserm and Institut National de Transfusion Sanguine, France; Irish  
143 Aid, Department of Foreign Affairs and Trade, Ireland.

144

145 **Keywords:** Malaria, *Plasmodium*, vaccine, VAR2CSA, pregnancy, placenta, First-in-  
146 Human, clinical trial.

147 **Research in context**

148 **Evidence before this study**

149 We systematically searched PubMed on June 4, 2019, for articles in English investigating the  
150 relationship between VAR2CSA and placental malaria (PM), using the terms (“VAR2CSA”  
151 [All Fields] AND “pregnancy” [All Fields] AND “malaria” [All Fields]) and also VAR2CSA  
152 and vaccine, using the terms (“VAR2CSA” [All Fields] AND “vaccine” [All Fields]). Our  
153 search returned respectively 182 and 106 articles, clearly indicative of a link between  
154 VAR2CSA and PM vaccine development. We also searched for articles about clinical trials of  
155 PM vaccines. We searched using the terms (“malaria” [All Fields] AND “vaccines” AND  
156 “placenta” [All Fields] AND “clinical trial” [All Fields]) OR (“malaria” [All Fields] AND  
157 “vaccines” AND “pregnancy” [All Fields] AND “clinical trial” [All Fields]) OR (“placental  
158 malaria vaccine” [All Fields] AND “clinical trial” [All Fields]). Only one recently published  
159 phase Ia study reporting the safety and immunogenicity of a VAR2CSA-derived placental  
160 malaria vaccine in malaria-naïve volunteers in Germany was identified. No clinical trial  
161 reporting the safety and immunogenicity of a placental malaria vaccine in a malaria endemic  
162 country was found.

163

164 **Added value of this study**

165 This is the first report of the safety and immunogenicity of a VAR2CSA-derived PM vaccine  
166 in both malaria naïve and *P. falciparum*-exposed non-pregnant women. PRIMVAC adjuvanted  
167 with either Alhydrogel® or GLA-SE delivered by intramuscular (IM) injection had an  
168 acceptable safety profile. All women vaccinated with PRIMVAC seroconverted after two  
169 vaccine doses and a high proportion of them were still seroconverted one year after the last  
170 immunization. Furthermore, PRIMVAC generated antibodies able to react with native  
171 VAR2CSA expressed on the surface of different strains and also inhibited the adhesion of the

172 homologous NF54-CSA strain to CSA. Limited cross-recognition, restricted to sera collected  
173 from women that have received the 100 µg PRIMVAC dosage, was observed against the FCR3-  
174 CSA and 7G8-CSA VAR2CSA-expressing parasites.

175

#### 176 **Implications of the available evidence**

177 The findings from this first-in-human clinical evaluation of the PRIMVAC vaccine in women  
178 in France and in Burkina Faso lays the foundation for further clinical evaluation. The advanced  
179 development of this PM vaccine candidate, alone or in combination with other PM vaccine  
180 candidates, now requires a deeper characterization of the breadth of vaccine-induced immune  
181 responses (humoral and cellular) upon natural exposure during pregnancy.

182

## 183 INTRODUCTION

184 According to the latest WHO's Malaria Report, 219 million cases of malaria occurred in 2017  
185 leading to 435 000 deaths.<sup>1</sup> The majority of clinical cases and deaths occurred in sub-Saharan  
186 Africa and were mainly resulting from *Plasmodium falciparum* infection. In malaria endemic  
187 areas, individuals progressively acquire clinical immunity during childhood and adults are  
188 therefore generally protected against the severe clinical outcomes of the disease.<sup>2</sup> However,  
189 during their first pregnancies, women become once again susceptible to the serious clinical  
190 outcomes associated with placental malaria (PM).<sup>3</sup> PM can lead to maternal anaemia,  
191 hypertension as well as stillbirth and low birth weight (LBW) due to premature delivery and  
192 foetal growth retardation.<sup>3</sup> LBW is a significant risk factor for neonatal and infant death.<sup>4</sup> A  
193 modelling study showed that up to 40% of pregnant women in sub-Saharan Africa develop  
194 PM.<sup>5</sup> Furthermore, *P. falciparum* malaria was responsible for 11% of LBW-related infant  
195 mortality in Sub-Saharan Africa<sup>6</sup> and an estimate of 217 026 stillbirths (20% of all stillbirths in  
196 sub-Saharan Africa).<sup>7</sup> Remarkably, the prevalence of PM sharply drops with successive  
197 pregnancies.<sup>4</sup> This protection has been associated with the development of antibodies directed  
198 towards the surface of infected erythrocytes (IEs) from placental origin.<sup>8</sup> Therefore, a vaccine  
199 priming the immunity observed in multigravid women could have a high impact on both disease  
200 incidence and severity and then save hundred thousand lives each year.<sup>3</sup>

201 The severe outcomes of PM results from the massive accumulation of IEs in the placental  
202 intervillous spaces<sup>2</sup> through the binding to the placental CSA, a binding phenotype not seen  
203 outside of the pregnancy context.<sup>9</sup> IEs adhesion is mediated by the highly diverse *P. falciparum*  
204 erythrocyte membrane protein family (PfEMP1) encoded by the *var* genes.<sup>2</sup>

205 Evidences strongly support the VAR2CSA-PfEMP1 variant as the leading candidate for a PM  
206 vaccine. Indeed, VAR2CSA is preferentially expressed by IEs from placental origin<sup>10</sup> and  
207 recombinant VAR2CSA binds to CSA.<sup>11</sup> Genetic deletion of *var2csa* results in the loss of IEs

208 adhesion to CSA that cannot be compensated by any other PfEMP1.<sup>12</sup> Anti-sera to recombinant  
209 VAR2CSA react to the surface of CSA-binding IEs and inhibit their adhesion to CSA<sup>13-15</sup>.  
210 Women gradually acquire strain-transcendent antibodies recognizing recombinant and native  
211 VAR2CSA expressed on IEs that also inhibit IEs adhesion to the placenta, thus correlating with  
212 PM protection.<sup>8,16,17</sup> Taken together, these data provide a rational basis for developing a  
213 VAR2CSA-derived PM vaccine. Two PM vaccine candidates (PRIMVAC and PAMVAC) are  
214 currently under clinical development.<sup>18</sup> Following an extensive screening study, PRIMVAC  
215 spanning the CSA-binding DBL1x-2x region of the 3D7-VAR2CSA variant was down-  
216 selected<sup>15</sup> and transitioned to current good manufacturing practice (cGMP) production.<sup>19</sup>  
217 Preliminary safety and immunogenicity results of the PAMVAC study, assessing a PM vaccine  
218 derived from the FCR3-VAR2CSA variant in malaria naïve volunteers in Germany, reported  
219 that PAMVAC is safe and can induce functional antibodies against the homologous  
220 VAR2CSA-expressing strain.<sup>20</sup>

221         Here, we report the safety and immunogenicity of PRIMVAC in both malaria naïve non-  
222 pregnant women in France and in *P. falciparum*-naturally exposed nulligravid women in  
223 Burkina Faso.

224

## 225 **METHODS**

### 226 **Study design and Participants**

227 This is a first-in-human phase Ia/Ib dose escalation trial, evaluating the safety and  
228 immunogenicity of three vaccinations with progressively higher dosages of PRIMVAC  
229 adjuvanted with Alhydrogel® or GLA-SE. A total of 68 healthy adult non-pregnant women  
230 were enrolled in four sequential cohorts. The trial started with two cohorts in malaria-naïve  
231 women in France (phase Ia, with 20 µg of PRIMVAC in cohort A, and then 50 µg of PRIMVAC  
232 in cohort B) and then pursued with two cohorts in nulligravid women living in Burkina-Faso  
233 (phase Ib, with 50 µg of PRIMVAC in cohort C, and then 100 µg of PRIMVAC in cohort D).  
234 Within each cohort, volunteers were randomized in a double-blind manner to two arms  
235 (PRIMVAC adjuvanted either with Alhydrogel® or GLA-SE) in phase Ia, and to three arms  
236 (PRIMVAC adjuvanted either with Alhydrogel® or GLA-SE or placebo) in phase Ib (**Table**  
237 **S1; appendix p10**). There was no placebo control arm in phase Ia. Enrolment into each cohort  
238 opened progressively following pre-defined rules after interim review of safety data and advise  
239 by an independent Data Safety Monitoring Board (DSMB). Full details of the study design are  
240 provided in the appendix pp 3-6. The protocol (N° EudraCT: 2015-002246-31) was approved  
241 by the French national Ethics Committee “CPP Ile de France III” (recorded under n°3328) and  
242 the French Medicine Agency (ANSM) (recorded under n°151347A-61) in France, and by the  
243 National Ethics Committee (CERS), the Institutional Bioethics Committee of the CNRFP (CIB-  
244 CNRFP) and the National Regulatory Authority (CTEC) in Burkina Faso. The trial was  
245 registered on ClinicalTrials.gov under the number NCT02658253 and was conducted in  
246 accordance with the Helsinki declaration.

247 Healthy non-pregnant women aged 18-35 years without any history of malaria and without  
248 recent travel or travel plans to malaria-endemic regions were eligible for enrolment in France.  
249 In Burkina Faso, healthy nulligest women aged 18-35 years were eligible, without any

250 restrictions with regards to prior *Plasmodium* infections or exposure. Volunteers were not  
251 eligible if they had previously received investigational malaria vaccines or if they intended to  
252 become pregnant during the trial. All participants provided written informed consent. Detailed  
253 eligibility criteria are provided in the appendix pp 3-5.

254

### 255 **Randomisation and masking**

256 French participants were randomized in a 1:1 ratio to receive PRIMVAC adjuvanted with  
257 Alhydrogel® or GLA-SE. In Burkina Faso, participants were randomized in a ratio of 1:2:2 to  
258 receive either placebo or PRIMVAC adjuvanted with Alhydrogel® or GLA-SE, respectively.  
259 The randomization sequence, using stratification by cohort and blocks of variable size, was  
260 computer generated with SAS software (version 9.3) by the unblinded statistician (EUCLID/F-  
261 CRIN Clinical Trials Platform, CIC1401, Bordeaux, France) and implemented in a validated  
262 web-based randomization tool (Clinsight® software). The participants were randomized by the  
263 investigator on their first vaccination visit (D0). Upon randomization, the Clinsight® software  
264 allocated the blinded treatment number via the electronic Case Report Form (eCRF). The  
265 correspondence list was made available at each site's pharmacy, where the unblinded  
266 pharmacist prepared and dispensed the masked vaccine syringe. All other site's staff and  
267 participants were masked to the treatment assignment.

268

### 269 **Procedures**

#### 270 **Vaccine candidate**

271 PRIMVAC expressed in *E.coli* SHuffle® strain was manufactured by Novasep Henogen  
272 (Gosselies, Belgium) on behalf of Inserm as the legal sponsor.<sup>19</sup> According to the randomized  
273 arm, volunteers received at days 0, 28 and 56, three intra-muscular (IM) injections of 20 µg, 50  
274 µg or 100 µg of PRIMVAC adjuvanted either with Alhydrogel® (0.85 mg aluminium content

275 per injection), manufactured by Brenntag (Denmark) or with GLA-SE (a stable oil-in-water  
276 emulsion containing glucopyranosyl lipid A (GLA), an investigational medicinal product (IMP)  
277 developed by the Infectious Disease Research Institute (IDRI, USA)).<sup>21</sup>  
278 GLA-SE was administered at 2.5 µg GLA in 2% oil per vaccine injection for cohorts receiving  
279 20 µg or 50 µg of PRIMVAC or at 2.56 µg GLA in 2% oil per vaccine injection for cohorts  
280 receiving 100 µg of PRIMVAC. Volunteers randomized to placebo in phase Ib received saline  
281 solution (NaCl 0.9%) (Chaix et du Marais, France). Preparation of the IMPs (PRIMVAC  
282 adjuvanted with Alhydrogel® or GLA-SE, or placebo) were performed by the pharmacist or an  
283 approved collaborator of the clinical site and delivered to the site study nurse or investigator in  
284 an individual masked syringe.

285

## 286 **Follow up**

287 In France, the volunteers were contacted by phone for the assessment of safety the day after  
288 each vaccination as well as 14 days later. To collect the adverse events following immunization  
289 (AEFI) in Burkina Faso, trained nurses under the supervision of the study clinicians paid a daily  
290 home visit to each enrolled study participant from days 1 to 7 after each vaccination. On-site  
291 safety visits took place seven days after each vaccination (at days 7, 35, and 63) and additional  
292 follow-up visits at days 90, 180 and 421. At each on-site visit, blood samples were collected  
293 for safety lab assessments at the local laboratories as well as for storage for later  
294 immunogenicity measurements. Between visits, volunteers recorded solicited local and  
295 systemic adverse events and any other symptoms in a diary booklet. Clinical data were collected  
296 and entered in the eCRF on site. Trial pausing rules were defined in the protocol in case of  
297 safety concerns (**appendix; p 6**).

298

## 299 **Immune response**

300 PRIMVAC total IgG antibody and isotypic subtypes antibody titres were measured by enzyme-  
301 linked immunosorbent assay (ELISA) (**appendix p8**). Data were fit to a 4-parameter sigmoidal  
302 curve, and the reciprocal serum dilution at which the optical density was 1·0 (OD1·0) for total  
303 IgG or 0·5 (OD0·5) for isotypic subtypes was calculated. Samples were considered positive if  
304 the difference between the post-immunization optical density (OD) 1·0 and the pre-  
305 immunization OD1·0 (net OD1·0) was >50 and the ratio of post-immunization OD1·0 to pre-  
306 immunization OD1·0 (ratio) was >2·5 as previously described.<sup>22</sup>

307

308 The cellular immune response was assessed *in vitro* by measuring production of the T-cell IL-  
309 2 and IL-5 cytokines by ELISPOT (Diaclone) after stimulation with the VAR2CSA antigen at  
310 a concentration of 10 µg/ml (**appendix pp 8-9**). A response was considered positive if the  
311 number of spots in the wells stimulated with the vaccine antigen was twofold higher than the  
312 number of spots in the negative control using a cut-off of 10 spot forming cells (SFC)/10<sup>5</sup> cells  
313 after background subtraction. In addition, the experiment was considered to be interpretable if  
314 the ELISPOT response to PMA-Ionomycin was ≥ 100 SFC for IL-2 and ≥ 10 SFC for IL-5,  
315 respectively.

316

317 *P. falciparum* laboratory adapted parasite strains NF54, FCR3 and 7G8 were grown and  
318 selected for the CSA-binding phenotype. These selected populations are referred to as NF54-  
319 CSA, FCR3-CSA and 7G8-CSA. Sera were assessed for reactivity against native VAR2CSA  
320 variants expressed on the surface of NF54-CSA, FCR3-CSA and 7G8-CSA IEs as previously  
321 described<sup>15</sup> using 50 µl of sera diluted (1:2) in PBS 1% BSA (**appendix p 9**). Results were  
322 expressed as the ratio of the geometrical mean fluorescence intensities (Geo. MFI) in the PE  
323 channel of the post-immunization samples over the respective pre-immunization samples.

324

325 Inhibition of IEs binding to CSA by sera collected at days 0, 35, 63 and 90 (1:5 dilution)  
326 previously decomplexed at 56°C for 45 minutes was assessed in a 96-well plate high  
327 throughput format as previously described<sup>19</sup>. Results were expressed as % of inhibition of the  
328 post-immunization samples compared to the respective pre-immunization samples [%  
329 inhibition = 100-(OD<sub>post-immunization</sub>/OD<sub>pre-immunization</sub>)/100].

330

### 331 **Outcomes**

332 The primary objective was to assess the safety of repeated PRIMVAC administration. The  
333 primary safety endpoint, also considered as the dose-escalation criterion by the DSMB, was the  
334 proportion of participants per cohort and randomized arm with any grade 3 and persisting at  
335 grade 3 for > 48 hours or higher clinical or laboratory adverse reaction to vaccination reported  
336 by the site investigator between day 0 and day 35. An adverse reaction was defined as an  
337 adverse event (AE) following immunization (AEFI) considered to be related or possibly related  
338 to the vaccine by the site investigator. AE were graded with the INYVAX EC FP7 Brighton  
339 Collaboration Foundation grading scale.<sup>23</sup> All events not covered by this scale were graded  
340 using the FDA scale, which was adapted for Burkina Faso taking into account the local  
341 reference ranges (**Table S2; appendix p 11**). Secondary safety endpoints included the number  
342 and proportion of AE and serious AE (SAE) at various time points after vaccination.

343 The secondary objective was to assess the PRIMVAC immunogenicity through 1) the humoral  
344 immune response (total IgG at D0, D28, D35, D56, D63, D90, D180 and D421 and isotypic  
345 subtypes at D0 and D90) and 2) the cellular immune responses after *in vitro* stimulation with  
346 the vaccine antigens (IL5 and IL2 secreting T cells) at D0, D7 and D63. Description of a binary  
347 response variable for seroconversion was added after initial description of the quantitative titres.  
348 Exploratory immunogenicity endpoints, measured at D0, D35, D63 and D90, concerned the  
349 capacity of the specific vaccine antibodies (IgG) to 1) recognize different VAR2CSA variants

350 expressed on the surface of parasitized erythrocytes and 2) to inhibit interactions between  
351 parasitized erythrocytes expressing different VAR2CSA variants and CSA.

352

### 353 **Statistical analysis**

354 Acknowledging that due to the small sample size, the power of phase I trials is in general very  
355 low, 10 participants per active arm in cohorts C and D constitute a trade-off between detectable  
356 event rate and power in the context of a phase I trial.<sup>24</sup> A sample size of 10 participants per  
357 active cohort arm in cohorts C and D allowed the observation of at least one SAE with 80%  
358 power if the underlying SAE rate is at least 15% (**appendix pp 6-8**).

359 Statistical analyses were performed per dose cohort and randomized arm and for the pooled  
360 placebo arms of cohorts C and D. A modified intention to treat (mITT) approach, including all  
361 participants having received at least one injection was used for the primary statistical analyses.  
362 Per protocol analyses were performed as additional analyses (**appendix pp 25-29**).

363 Proportions of the primary endpoint were described with 90% two-sided binomial confidence  
364 intervals. Geometric means and their two-sided 95% confidence interval were used to describe  
365 antibody titres. To evaluate the magnitude of the humoral (total IgG) and cellular responses  
366 (IL2 and IL5 secreting T cells), intra-group comparisons were performed using the Wilcoxon  
367 paired signed-rank test with a two-sided significance level of 5%. No statistical comparisons  
368 between groups or cohorts were done as this phase I trial was not designed for such  
369 comparisons. Interim safety reviews by the independent DSMB prior to dose escalation were  
370 based on descriptive analyses. All analyses were performed using SAS software (version 9.4).

371

### 372 **Role of the funding source**

373 Inserm was the sponsor of the study. This study has been funded by the European Vaccine  
374 Initiative (EVI) (via funds from German Ministry for Education and Research (BMBF) through

375 Kreditanstalt für Wiederaufbau (KfW) and Irish Aid, Department of Foreign Affairs and Trade,  
376 Ireland), Inserm and Institut National de Transfusion Sanguine. EVI and Inserm representatives  
377 were members of the Trial Steering Committee and as such were involved in the study design,  
378 the overview of the study conducts and analyses, the writing of the report and in the decision  
379 to submit the manuscript for publication.

380

381 **RESULTS**

382 Between April 19, and July 5, 2016, 25 women were screened and 18 were included in cohorts  
383 A and B in France (**Figure 1**). Six participants were first assigned to receive 20 µg of  
384 PRIMVAC in combination with either Alhydrogel® (n=3) or GLA-SE (n=3). The 12 other  
385 participants were assigned to receive 50 µg of PRIMVAC/Alhydrogel® (n=6) or 50 µg of  
386 PRIMVAC/GLA-SE (n=6). Between November 25, 2016 and July 13, 2017, 76 women were  
387 screened and 50 were included in cohorts C and D in Burkina Faso (**Figure 1**). Of the 49 women  
388 screened for cohort C, 25 were included and randomized to receive 50 µg dosage of  
389 PRIMVAC/Alhydrogel (n=10) or PRIMVAC/GLA-SE (n=10) or placebo (n=5). Of the 27  
390 women screened for cohort D, 25 were included in each cohort and randomized to receive 100  
391 µg dosage of PRIMVAC/Alhydrogel (n=10) or PRIMVAC/GLA-SE (n=10) or placebo (n=5).  
392 The main characteristics of the enrolled participants in each cohort and randomized arm are  
393 described in **Table 1**.

394 During the trial, after review of the safety data, the DSMB recommended opening of the cohorts  
395 as planned by the protocol. Pregnancy of one woman in cohort B was discovered immediately  
396 after randomization and she thus did not receive any injection and was excluded from the mITT  
397 analyses. Among the 67 volunteers included in the analyses, 63 received all three injections. In  
398 cohort C, due to a pregnancy, one woman in the GLA-SE group did not receive the third  
399 vaccination. In cohort D, one woman in the placebo group and one woman in the GLA-SE  
400 group did not receive the third vaccination and one woman in the placebo group did not receive  
401 the second and the third vaccinations (withdrawal of consent).

402 Participants who received PRIMVAC at all dosage and adjuvant combinations had a good  
403 safety profile, with most AE being of grade 1 or grade 2 (**Table 2 and Table S3; appendix pp**  
404 **12-13**). No grade 3 or grade 4 clinical or biological AE related to the vaccine was observed  
405 during the trial. A total of 338 AE and 186 adverse events following immunization (AEFI)

406 related to the vaccine were documented (**Table 2 and Table S3; appendix pp 12-13**). For the  
407 cohort A (n=6), 54 AE were reported, 45 related to the vaccine: 25 in the Alhydrogel<sup>®</sup> group  
408 (grade 1[n=20]; grade 2[n=5]) and 20 in the GLA-SE group (grade 1[n=16]; grade 2 [n=4]).  
409 For the cohort B (n=11), 105 AE were reported, 84 related to the vaccine (82 clinical and 2  
410 biological): 40 clinical AE in the Alhydrogel<sup>®</sup> group (grade 1 [n=21]; grade 2 [n=19]) and 42  
411 in the GLA-SE group (grade 1[n=28]; grade 2 [n=14]). Two SAE (meningeal syndrome grade  
412 3 n=1 and anemia grade 3 n=1) were reported in the GLA-SE group of cohort B, 7.5 and 14  
413 months respectively after the last vaccine injection and were considered not related to  
414 vaccination. For the cohort C (n=25), 94 AE were reported, 33 related to the vaccine: 18 in the  
415 Alhydrogel<sup>®</sup> group (grade 1[n=14] or 2 [n=4]), and 10 in the GLA-SE group (grade 1 [n=9] or  
416 2 [n=1]) and 5 in the placebo group (grade 1 [n=5]). For the cohort D (n=25), 85 AE were  
417 reported, 24 related to the vaccine: 15 in Alhydrogel<sup>®</sup> group (grade 1[n=15]) and 9 in the GLA-  
418 SE group (grade 1[n=8] or 2 [n=1]). Thirty cases of serious biological AE (hyponatremia  
419 [n=27], hypoprotidemia [n=1] and hyperkalaemia [n=2]), were reported in Burkina Faso  
420 (cohort C and D). None were related to the vaccine or to the study procedure. Hyponatremia  
421 events were attributed to measurement errors due to a malfunction of the electrolyte analyzer.  
422 No adverse pregnancy outcomes have been reported for the pregnancies occurring in trial  
423 participants.

424 The most common vaccine related AE (N=186) were injection site-related events (n=123  
425 [66.1%]), asthenia (n=19 [10.2%]), and headache (n=18[9.7%]). The most frequent local  
426 reactions were pain at injection site and limitation of arm motion abduction (**Table 3**).

427 The primary safety endpoint (proportion of participants per cohort with any grade 3 and  
428 persisting at grade 3 for > 48 hours or higher clinical or laboratory adverse reaction to  
429 vaccination reported by the site investigator between day 0 and day 35) was 0% in all cohorts  
430 and arms, except for the pooled placebo arm, in which this proportion was estimated at 10%

431 (N=10) (**Table S4; appendix p 14**). This was not due to an observed adverse event but to the  
432 fact that the endpoint observation was missing for one volunteer who withdrew her consent  
433 prior to day 35 and who was imputed as a safety “failure” in the statistical analysis (analysis  
434 strategy missing=failure).

435

436 Antibodies titres to PRIMVAC were assessed by ELISA on sera collected on vaccination days  
437 (D0, D28 and D56), seven days after the second and third vaccinations (D35 and D63) as well  
438 as 90, 180 and 421 days after the first vaccination. No PRIMVAC antibody titre above 100 was  
439 detected in any of the volunteers in cohorts A and B before vaccination. Sixteen women from  
440 cohorts C and D had detectable levels of antibodies before vaccination (dilution above 1/100 to  
441 reach an OD of 1.0) (maximum geometric mean titre encountered at baseline: 32.6 OD1.0  
442 [95%CI 6.7-158.6]) (**Figure 2 and Table S5; appendix p15, Figure S1; appendix p22**).

443 Antibody titres to PRIMVAC increased with each dose with the highest levels usually observed  
444 7 days after the third vaccination (D63) (**Figure 2 and Table S5; appendix p 15, Figure S1;  
445 appendix p22**). Placebo recipients (cohorts C and D) did not have any marked antibody  
446 increases over time. Positive antibody responses were observed in 56 out of 57 PRIMVAC-  
447 vaccinated women 28 days after the second injection (D56) (**Table S6; appendix p 16**).  
448 Seroconversion could not be assessed at D56 and D63 for one volunteer (cohort D GLA-SE)  
449 who did not come to the planned visits and then did not receive the third vaccination.  
450 Interestingly, although having received only two vaccinations, she had a positive antibody  
451 response at D90 and up to the last visit (D421). A trend for higher geometrical means was  
452 observed for all the cohorts vaccinated with PRIMVAC adjuvanted with GLA-SE compared to  
453 Alhydrogel<sup>®</sup> (geometric means at D63, 5997.7 OD 1.0 [95% CI 4208.9-8546.6] and 1581.7  
454 [95% CI 925.0-2 704.4] respectively for cohort C PRIMVAC 50µg; 11843.0 [95% CI 7 559.8-  
455 18552.9] and 2163.5 [95% CI 1 315.7-3 557.7] respectively for cohort D PRIMVAC 100µg)

456 **(Figure 2 and Table S5; appendix p 15 and Figure S1; appendix p22)**. The highest antibody  
457 level was reached one week after the third vaccination (Day 63) in the 100 µg PRIMVAC/GLA-  
458 SE cohort (geometric mean 11843 OD 1.0, 95% CI 7559.8-18552.9). Antibody titres decreased  
459 gradually in all PRIMVAC-vaccinated women between D63 and D421. Four months (D180)  
460 after the last vaccination, 54 out of the 56 (96.4%) PRIMVAC-vaccinated women still had  
461 positive PRIMVAC specific antibody responses, while one-year (D421) after the last  
462 vaccination, 20 out of 28 (71.4%) PRIMVAC/Alhydrogel<sup>®</sup> and 26 out of 28 (92.8%)  
463 PRIMVAC/GLA-SE vaccinated women still had positive responses **(Table S6; appendix p**  
464 **16)**. Although two volunteers of the higher PRIMVAC/GLA-SE dosage (Cohort D) were no  
465 longer considered responders at D421, this was due to high antibody titers at D0 and they still  
466 possessed PRIMVAC specific antibodies.

467 PRIMVAC isotypic subtypes antibody titres were determined at D0 and 34 days after the third  
468 vaccination (D90). Most of the induced antibodies were IgG1, followed by IgG3 in the  
469 PRIMVAC/GLA-SE vaccinated volunteers in cohorts C and D (geometric mean 485.7 OD 0.5  
470 [95% CI 351.1-671.9] for IgG1 and 43.6 [95% CI 22.8-83.6] for IgG3 at D90 for PRIMVAC  
471 100 µg) **(Table S7; appendix p17)**. No IgG2 and IgG4 were detected in any of the  
472 dosage/adjuvant combinations.

473 Following PRIMVAC immunization, an increase in the antigen specific IL-2 and IL-5  
474 producing T cell responses was observed at D63 compared to D0 across cohorts (maximum  
475 median increase: +36.5 (interquartile range: 18.0-41.0) SFC/10<sup>5</sup> observed for IL-2 in  
476 PRIMVAC 50 µg with GLA-SE) **(Figure S2 appendix p 23 and Table S8; appendix p 18)**.

477 The ELISPOT responses tended to be greater with GLA-SE than with the Alhydrogel<sup>®</sup>  
478 adjuvant. No increase in the T cell response was observed with increasing vaccine doses.

479 To assess the capacity of vaccine-induced antibodies to recognize native VAR2CSA on the IEs  
480 surface, sera samples were co-incubated with NF54-CSA, FCR3-CSA and 7G8-CSA purified

481 IEs. Flow cytometry analysis revealed that the homologous VAR2CSA expressing NF54-CSA  
482 IEs were recognized by total serum IgGs from all participants from cohorts C and D as soon as  
483 7 days after the second vaccination (D35) (Cohort D fold changes from baseline at D35 with  
484 PRIMVAC 100 µg : 2.29 [95% CI 1.55-3.38] for GLA-SE and 1.52 [95% CI 1.10-2.08] for  
485 Alhydrogel) (**Figure 3 and Table S9; appendix p 19**). Cell surface labelling was confirmed  
486 by confocal microscopy (**Figure S3; appendix p 24**).

487 At Day 35, NF54-CSA IEs recognition was weaker with samples from cohorts A and B (Cohort  
488 B fold changes from baseline: 1.47 [95% CI 0.83-2.59] for GLASE and 1.04 95% CI [0.94-  
489 1.15] for Alhydrogel) and barely detectable when considering the 2 arms adjuvanted with  
490 Alhydrogel®. Nevertheless, 7 and 30 days after the third vaccination (D63 and D90) all  
491 PRIMVAC-vaccinated volunteers possessed antibodies able to react with NF54-CSA IEs,  
492 reaching up to a 15-fold increase in Geo. MFI as compared to D0 for participants vaccinated  
493 with 20 µg PRIMVAC in combination with GLA-SE. Less recognition was observed against  
494 the native VAR2CSA expressed on both FCR3-CSA and 7G8-CSA IEs. Indeed, at D35, none  
495 of the PRIMVAC-vaccinated women had relevant antibodies cross-reacting with native  
496 VAR2CSA expressed at the surface of FCR3-CSA and 7G8-CSA IEs. Compared to D0, an  
497 increased reactivity was observed at D63 and D90 for the highest PRIMVAC dose (Cohort D)  
498 adjuvanted with both Alhydrogel® and GLA-SE (up to 1.49-fold increase in Geo. MFI [95%  
499 CI 1.19-1.88] in the GLA-SE arm against FCR3-CSA at D63) (**Table S9; appendix p 19**).

500

501 The capability of vaccine-induced antibodies to inhibit the adhesion of VAR2CSA-expressing  
502 IEs to the placental receptor CSA was assessed. While inhibition of the interaction of NF54-  
503 CSA IEs with CSA was absent or weak in all cohorts at D35, (**Figure 4 and Table S10**  
504 **appendix p 20**), blocking activity was observed at D63 and D90 for all cohorts. The strongest  
505 blocking effect was observed 7 days after the third vaccination (D63) in the PRIMVAC/GLA-

506 SE cohort D (mean 58.3% inhibition, 95% CI 48.5-68.1). Low or no CSA-binding inhibition  
507 was observed on FCR3-CSA or 7G8-CSA IEs expressing heterologous VAR2CSA variants  
508 **(Table S10; appendix p 20).**

509 All the conclusions regarding the immune responses based on per-protocol analyses **(Table**  
510 **S12, Figures S4-S6, appendix p 25-28)** were consistent with those from the ITT analyses.

511

512 **DISCUSSION**

513 VAR2CSA-based PM vaccines administered before first pregnancy stand as the main anti-  
514 disease strategy to reduce malaria morbidity and mortality<sup>18</sup>. Here, we report the results of a  
515 phase Ia/Ib clinical trial, assessing the safety and immunogenicity of the PRIMVAC PM  
516 vaccine. PRIMVAC adjuvanted with either Alhydrogel<sup>®</sup> or GLA-SE presented an acceptable  
517 safety profile, as only grade 1 and 2 AEs related to the vaccine were reported. All women  
518 vaccinated with PRIMVAC seroconverted after two vaccine doses and antibody titres reached  
519 a peak one week after the third vaccination (Day 63). Although PRIMVAC adjuvanted with  
520 either Alhydrogel<sup>®</sup> or GLA-SE was immunogenic in all cohorts, GLA-SE appeared more potent  
521 than Alhydrogel<sup>®</sup> at inducing IgG responses. This higher response is in line with the results  
522 obtained in the phase Ia PAMVAC study, which assessed the safety and immunogenicity of a  
523 FCR3-VAR2CSA vaccine in malaria naïve volunteers.<sup>20</sup> With respect to each adjuvant, no  
524 clear dose-response relationship in terms of IgG response amplitude was observed between the  
525 PRIMVAC dose 20 µg and 50 µg in France or between the dose 50 µg and 100 µg in Burkina  
526 Faso. However, the geometric mean PRIMVAC-specific antibody titres in the 50 µg cohorts  
527 tended to be higher in women from Burkina Faso than in French women. In the context of PM,  
528 the emergence of VAR2CSA expressing IEs during natural *P. falciparum* infection is made  
529 possible by the appearance of the placenta. Thus, theoretically nulligravid women should not  
530 have encountered VAR2CSA expressing IEs to naturally develop an immune response toward  
531 the antigen. This is generally reflected by a low VAR2CSA-recognition at baseline. However,  
532 out of 50 Burkinabe women, 16 possessed PRIMVAC-specific IgG titres > 100 before  
533 vaccination. Field studies have previously reported low levels of anti-VAR2CSA antibodies in  
534 children, men and teenage girls.<sup>25-27</sup> Therefore, antibodies to VAR2CSA seems to be acquired,  
535 to some extent, outside of the pregnancy context. This could be the consequence of prior  
536 exposure to VAR2CSA or to malaria antigens sharing common epitopes with VAR2CSA such

537 as other PfEMP1 or *P. vivax* Duffy Binding Protein (PvDBP).<sup>28</sup> The higher antibody titres  
538 observed in Burkinabe women may thus result from a recall of pre-existing immunological  
539 responses.

540 Highest anti-PRIMVAC IgG levels were seen at the end of vaccination and were accompanied  
541 by a release of cytokines by T-cells. Both IL-2 and IL-5 responses tended to be greater with  
542 GLA-SE than with Alhydrogel<sup>®</sup>. Notably, the median of SFC in the IL-2 ELISPOT seems to  
543 be lower in the malaria pre-exposed women than in the malaria naive women. IL-2-mediated  
544 differentiation of T cells into T<sub>helper</sub> cells is essential for B cell antibody class switching, and the  
545 concomitant secretion of IL-5 will stimulate B cells to produce antibodies. Thus, PRIMVAC  
546 seems to orientate the immune system towards a mixed Th1/Th2 response, which is favourable  
547 for vaccine development.

548 VAR2CSA sequence polymorphism represents a major hurdle for PM vaccine development.  
549 Efforts have been deployed to design VAR2CSA-based polypeptides able to generate  
550 antibodies cross-recognizing semi-conserved antigenic determinants present within different  
551 VAR2CSA variants. Our previous pre-clinical studies highlighted that conserved epitopes  
552 within VAR2CSA variants were present in restricted numbers and/or were non-  
553 immunodominant<sup>13,15,19</sup>. In line with these pre-clinical studies, PRIMVAC was able to generate  
554 antibodies reacting with VAR2CSA expressed on the surface of the homologous NF54-CSA  
555 strain as soon as 7 days following the second PRIMVAC injection, with a more pronounced  
556 effect in both African cohorts, thus indicating that these antibodies are produced at early time-  
557 points of the vaccination schedule. However, low cross-recognition of VAR2CSA variants  
558 expressed by FCR3-CSA and 7G8-CSA strains was only detected in sera collected at Days 63  
559 and 90 from women who received the 100 ug dose. The weaker recognition amplitude observed  
560 for FCR3-CSA and 7G8-CSA IEs than for NF54-CSA suggests that only a restricted number  
561 of conserved accessible epitopes exists between the different VAR2CSA variants and/or that

562 antibodies targeting these conserved epitopes are present in low amounts due to their weak  
563 immunogenicity.

564 The vaccine-induced antibodies were also able to inhibit the adhesion of the homologous  
565 VAR2CSA-expressing NF54-CSA IEs to CSA with the higher activity being observed in the  
566 100 µg PRIMVAC dosage adjuvanted with GLA-SE. However, no inhibition of adhesion was  
567 observed on the heterologous FCR3-CSA and 7G8-CSA strains. This lack of inhibition is likely  
568 the consequence of the low cross-reactivity observed by flow cytometry.

569 These observations raise an important question regarding the cross-reactive antibody threshold  
570 required for protection. PM protection has been correlated with the capacity of antibodies to  
571 recognize VAR2CSA-expressing IEs and inhibit their interaction with CSA. However, little is  
572 known about other potential protective modes of action and required levels of anti-VAR2CSA  
573 antibodies. Furthermore, the importance and the respective role of other antibody-mediated  
574 immune effector mechanisms such as Antibody Dependent Cell-mediated cytotoxicity (ADCC)  
575 and Antibody Dependent Phagocytosis (ADP) remain unclear.

576 IgG sub-classes analysis performed 34 days after the last vaccination revealed that most of the  
577 induced antibodies were IgG1 and IgG3, both sub-classes being able to interact with most Fc  
578 receptors present on immune effector cells. Interestingly, the vaccine-induced antibody  
579 response seems to mimic the naturally acquired immune response observed in previous  
580 observational studies showing that IgGs from multigravida women reactive to placental parasite  
581 isolates were mainly IgG1 and to a lesser extent IgG3 <sup>29,30</sup> and that both sub-classes correlated  
582 with the ability of serum or plasma to inhibit adhesion of IEs to CSA <sup>30</sup>. A recent manuscript  
583 have shown that IgM is an important functional and long-lived antibody response targeting  
584 blood-stage malaria parasites that contributes to malaria immunity <sup>31</sup>. Therefore, it could be  
585 interesting in the future to assess IgM vaccine induction.

586 Evaluation of vaccine efficacy remains particularly complex for malaria. To date, the lack of  
587 surrogates to predict vaccine-induced protection against PM limits the potential of early clinical  
588 trials to provide hints for possible vaccine efficacy. The amplitude of the humoral response  
589 resulting from vaccine administration appears today as a promising indicator of potential  
590 vaccine efficacy, even though the threshold levels of antibodies required for protection are  
591 undetermined. Numerous studies correlated the presence of anti-VAR2CSA antibodies with  
592 protection against PM.<sup>2,8,16</sup>

593 PRIMVAC aims at priming the immunity of nulligravid women against placental-type IEs.  
594 Interestingly, the 3D7-VAR2CSA PRIMVAC appears here as a vaccine candidate of choice  
595 since a recent study suggested that women infected with 3D7-like variants deliver infants with  
596 lower birthweight as compared to women infected with FCR3-like variants.<sup>32</sup>

597 In the field, pregnant women are exposed to a variety of parasites harbouring different  
598 genotypes and therefore displaying different VAR2CSA variants. The natural boosts of  
599 PRIMVAC vaccinated-individuals could then enhance the response against conserved epitopes  
600 and consequently accelerate the induction of a durable clinical protection. Therefore, although  
601 not planned initially, it seems important to follow the vaccinated Burkinabe women in order to  
602 assess the sustainability, longevity and potential expansion of the PRIMVAC induced humoral  
603 response in the context of natural exposure and also in the context of future pregnancies. This  
604 follow-up could provide some indication whether or not the immune response associated to  
605 PRIMVAC could help these women in expanding an immune response likely to protect against  
606 PM.

607 In parallel and in order to enhance the PRIMVAC induced immune response, other schedule of  
608 immunization, antigen dosage and adjuvant combinations could be assessed in future studies.  
609 Alternative strategies could also be explored, such as using other delivery platform, combining

610 PRIMVAC with other VAR2CSA derived vaccines or using heterologous prime-boost  
611 regimens incorporating different variants of the VAR2CSA CSA-binding region.

612

613 In conclusion, PRIMVAC presented an acceptable safety profile and was immunogenic in both  
614 women never exposed to *P. falciparum* in France and nulligravid women living in a malaria  
615 endemic area in Burkina Faso. Seroconversion was observed in all PRIMVAC-vaccinated  
616 women and the combination with GLA-SE tends to increase PRIMVAC-specific IgG levels  
617 and duration of the response. However, only the higher dosage was able to induce cross-reactive  
618 antibodies against other VAR2CSA variants, and no cross-inhibition was observed.

619 Future studies should seek to increase the level of cross-reactive and cross-inhibitory  
620 antibodies. Follow-up of the Burkinabe women vaccinated with PRIMVAC could provide some  
621 indication whether or not the immune response associated to PRIMVAC vaccination could help  
622 these women in accelerating a clinical protection against PM.

623

624 **FIGURE LEGENDS**

625 **Figure 1: Trial profile.** Eighteen volunteers were enrolled in cohorts A and B in France and 50 volunteers were  
626 enrolled in cohorts C and D in Burkina Faso. Opening of the cohort B was scheduled if no adverse events following  
627 immunization was reported at D7 after the first vaccination in any of the six volunteers of this cohort. Opening of  
628 cohorts C and D was realized after safety data review by the DSMB. Among the volunteers, 63 received all three  
629 injections of PRIMVAC.

630  
631 **Figure 2. Anti-PRIMVAC IgG ELISA titres.** Total IgG response to the PRIMVAC antigen was assessed by  
632 Enzyme Linked Immuno-Sorbent Assay (ELISA) at D0, 28, 35, 56, 63, 90, 180 and D421 in cohorts A and B in  
633 France (A) and in cohorts C and D in Burkina Faso (B). Data were fit to a 4-parameter sigmoidal curve, and the  
634 reciprocal serum dilution at which the optical density was 1.0 (OD1.0) was calculated. Closed circles represent  
635 geometric mean antibody titres (GMT) and black bars show 95% confidence intervals (CI).  
636

637 **Figure 3. Immune recognition of native VAR2CSA expressed on the surface of IEs.** Erythrocytes infected by  
638 the VAR2CSA expressing parasites strain NF54-CSA, FCR3-CSA and 7G8-CSA were incubated with individual  
639 serum samples from cohorts A and B (A) and C and D (B) at D0, 35, 63 and D90. Erythrocyte-bound IgGs were  
640 detected using an anti-human IgG PE-conjugated antibody. Cells were then subjected to flow cytometry analysis.  
641 Results are expressed as the fold-change in geometrical mean fluorescence intensity (PE) between the post-  
642 immunization samples and the respective pre-immunization samples. Results are depicted with 95% confidence  
643 interval (CI) levels.  
644

645 **Figure 4. Inhibition of IEs binding to the placental receptor CSA by vaccination-induced antibodies.**  
646 Erythrocytes infected by the autologous parasite strain NF54-CSA were pre-incubated with individual serum  
647 samples from cohorts A and B (A) and C and D (B) at D0, 35, 63 and D90. CSA-binding inhibition was assessed  
648 by relative quantification of IEs remaining bound to the plate surface after washes. For a given condition, the  
649 percentage of inhibition was calculated as follow: [% inhibition =  $100 - (\text{OD}_{\text{post-immunization}} / \text{OD}_{\text{pre-immunization}}) / 100$ ].  
650 Results are depicted with 95% confidence interval (CI) levels.  
651

652

653 **CONTRIBUTORS**

654

655 SBS, Ola and BG were the principal investigators. SBS, LR, AC, ATK, OLe, PL, IN, VB, GR,  
656 ELP, AK, EK, HE, FB, ET, NKV, RT, OLa and BG designed the trial. SBS, LR, AC, ATK,  
657 SD, JPS, NB, MB, PL, AIO, IN, MoK, DK, AB, SMO, VB, FA, GR, ELP, AK, EK, CR, MyK,  
658 LB, AD, NH, IS, AO, HE, Ola, BG acquired and curated the data. AC, ATK, SD, JPS, NB,  
659 MB, IN, MoK, DK, AO, FB, ET performed the laboratory experiments. AC, NH, NKV and BG  
660 developed the vaccine. LR, CC, and RT performed the statistical analysis. SBS, LR, AC, ATK,  
661 CC, CR, SB, JPS, NB, MB, AIO, IN, VB, FB, ET, NKV, RT, OLa and BG interpreted data and  
662 results. SBS, LR, AC, CC, RT, OL and BG wrote the first version of the manuscript. All authors  
663 critically reviewed and validated the final version of the manuscript.

664

665 **DECLARATION OF INTERESTS**

666 SBS, AC, ATK, AIO, IN, MoK, DK, AB, SMO, NH, IS, AO, Ola and BG report grants from  
667 European Vaccine Initiative, during the conduct of the study.

668 LR report grants from European Vaccine Initiative, from French Clinical Research  
669 Infrastructure Network (F-CRIN) (funds from ANR, France) for Clinical Trials Unit core  
670 structure, during the conduct of the study.

671 NKV and Ole report grants from Bundesministerium für Bildung und Forschung (BMBF),  
672 Germany through Kreditanstalt für Wiederaufbau (KfW), grants from Irish Aid, Department of  
673 Foreign Affairs and Trade, Ireland, during the conduct of the study; grants, personal fees, non-  
674 financial support and other from Pharmaceutical and drug discovery companies, outside the  
675 submitted work.

676 NH reports grants from Bundesministerium für Bildung und Forschung (BMBF), Germany  
677 through Kreditanstalt für Wiederaufbau (KfW), grants from Irish Aid, Department of Foreign  
678 Affairs and Trade, Ireland, during the conduct of the study; grants, personal fees, non-financial  
679 support and other from European Vaccine Initiative, outside the submitted work.

680

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**Table 1 –Characteristics of the vaccinated participants**

	<b>Cohort A:</b>		<b>Cohort B:</b>		<b>Cohort C:</b>		<b>Cohort D:</b>		<b>Placebo</b>	<b>Total</b>
	<b>PRIMVAC 20µg</b>		<b>PRIMVAC 50µg</b>		<b>PRIMVAC 50µg</b>		<b>PRIMVAC 100µg</b>			
	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE		
	n=3	n=3	n=5	n=6	n=10	n=10	n=10	n=10	n=10	n=67
Age, years	21	26	30	28	21	20	19	18	19	20
	(20 ; 35)	(24 ; 34)	(27 ; 32)	(27 ; 31)	(20 ; 21)	(19 ; 21)	(18 ; 20)	(18 ; 19)	(18 ; 21)	(18 ; 22)
BMI , kg/m <sup>2</sup>	22.5	21.1	21.4	23.1	19.9	20.8	21.1	20.0	23.3	21.1
	(21.5 ; 23.6)	(21.0 ; 23.9)	(19.8 ; 25.3)	(20.1 ; 27.7)	(19.0 ; 20.6)	(20.4 ; 21.3)	(20.3 ; 22.2)	(19.5 ; 22.0)	(21.4 ; 25.1)	(19.8 ; 23.7)

Median and interquartile range are shown

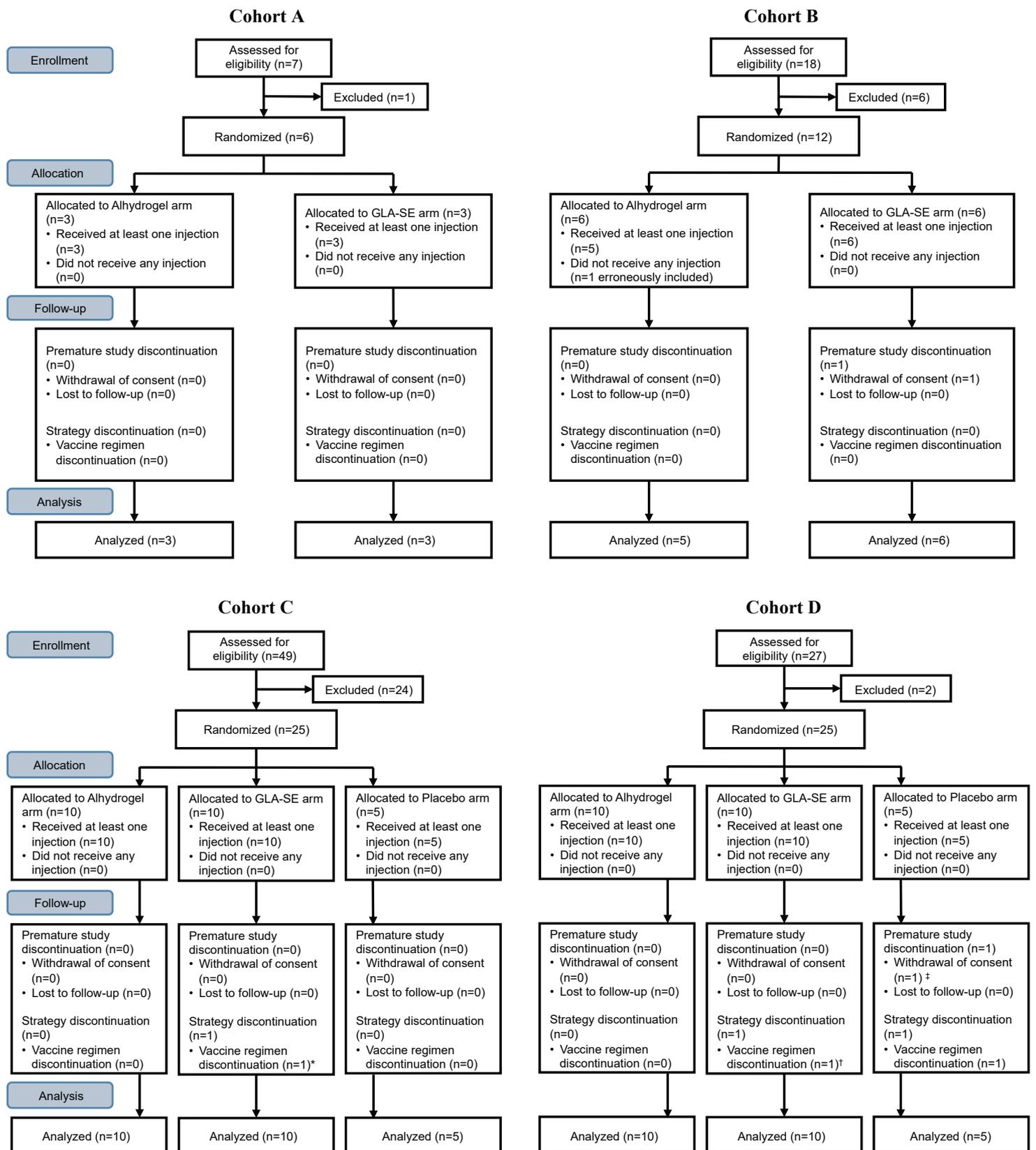


**Table 3 – Number of adverse events related to vaccination by MedDRA System Organ Class (SOC) and Preferred Term (PT)**

		Cohort A		Cohort B		Cohort C		Cohort D		Placebo									
		Alhydrogel	GLA-SE	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE										
<b>SOC (and PT), n (%)</b>	<b>Maximal Grade</b>	n=25		n=20		n=42		n=42		n=18		n=10		n=15		n=9		n=5	
<b>General disorders and administration site condition</b> Injection site reaction: pain, movement impairment, erythema, induration, swelling, hypoesthesia, haematoma, inflammation, node, oedema, pruritus, asthenia, fatigue, chills	Grade 1	18	(72)	14	(70)	18	(43)	22	(52)	13	(72)	7	(70)	13	(87)	7	(78)	1	(20)
	Grade 2	5	(20)	3	(15)	11	(26)	11	(26)	3	(17)								
<b>Nervous system disorders</b> Headache	Grade 1	2	(8)	1	(5)			1	(2)	1	(6)	1	(10)	1	(7)	1	(11)	4	(80)
	Grade 2			1	(5)	4	(10)									1	(11)		
<b>Musculoskeletal and connective tissue disorders</b> Arthralgia myalgia, dorsalgia	Grade 1					1	(2)	3	(7)										
	Grade 2					2	(5)	2	(5)	1	(6)	1	(10)						
<b>Gastrointestinal disorders</b> Nausea, diarrhea, dyspepsia	Grade 1			1	(5)	2	(5)	2	(5)			1	(10)	1	(7)				
	Grade 2					1	(2)												
<b>Investigations</b> ALAT increased, ASAT increased	Grade 1					1	(2)												
	Grade 2					1	(2)												
<b>Psychiatric disorders</b> Irritability	Grade 2					1	(2)												
<b>Vascular disorders</b> Hot flush	Grade 2							1	(2)										

Version 20.0 of MedDRA was used for coding adverse events

Figure 1



\* Pregnancy, D56 (injection visit) not done

† D56 (injection visit) not done

‡ D28 and D56 (injection visit) not done

Figure 2

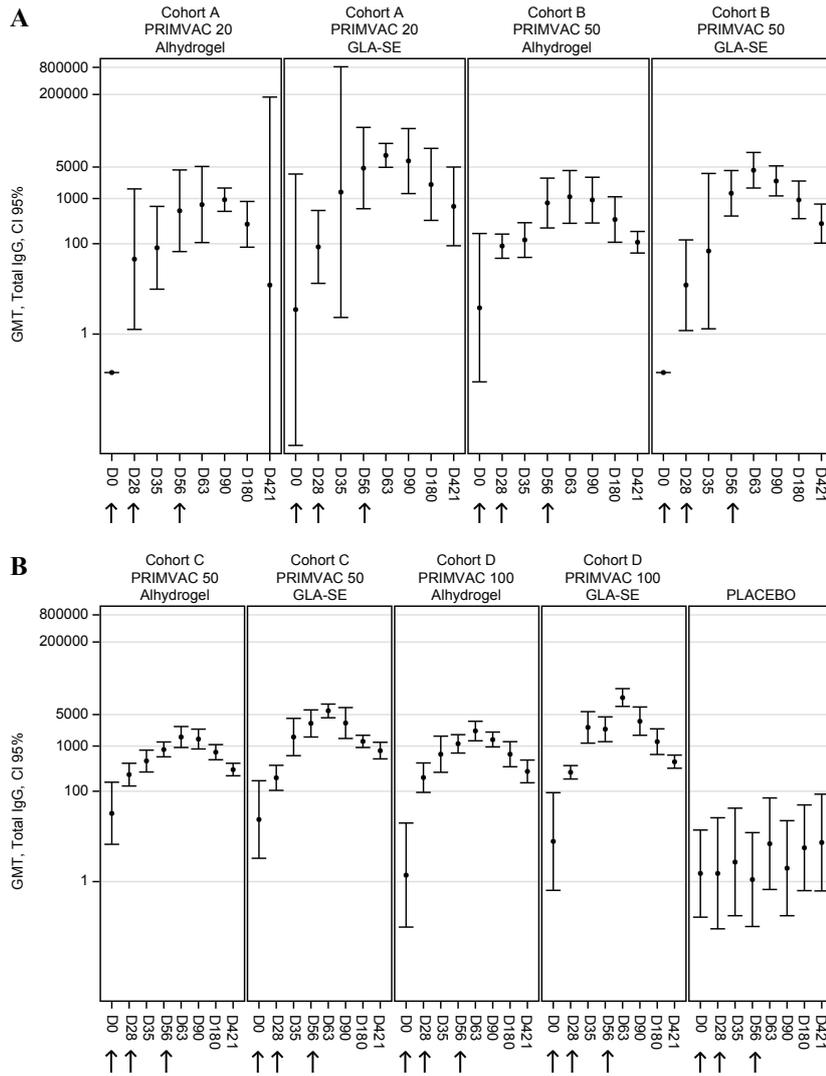


Figure 3

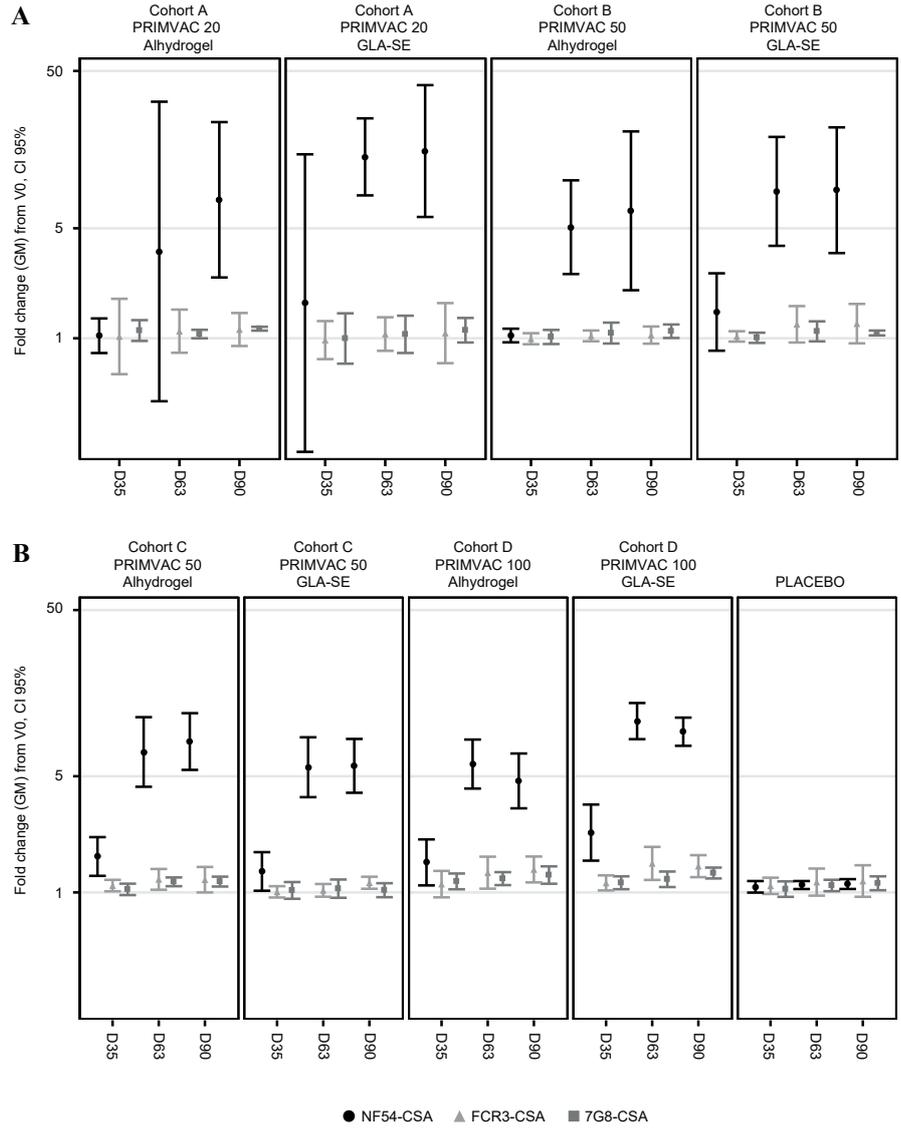


Figure 4

