Plasmodium falciparum and Necator americanus in Gabon: observation, intervention, and controlled human infection

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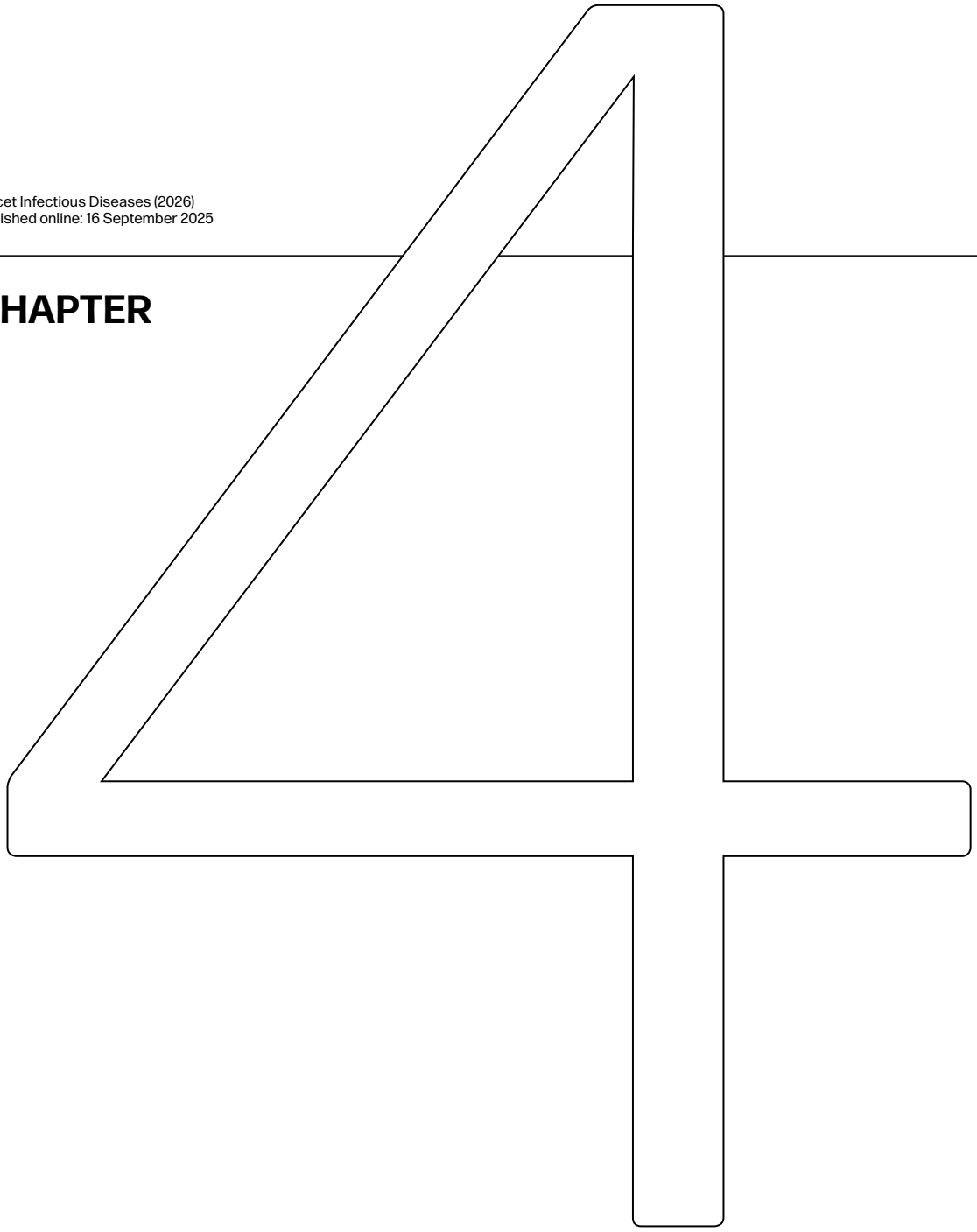
CHAPTER



Safety, tolerability, and protective efficacy of a radiation-attenuated, whole sporozoite malaria vaccine in children in Gabon: a randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background Highly effective malaria vaccines are crucial to further reduce the burden of malaria. The radiation-attenuated *Plasmodium falciparum* sporozoite (*Pf*SPZ) Vaccine protects adults; however, there are insufficient efficacy data in child populations. We aimed to assess the safety and efficacy of the *Pf*SPZ Vaccine in children aged 1–12 years in Gabon.

Methods This randomised, double-blind, placebo-controlled, phase 2 trial was conducted at the Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon. Healthy children were stratified by age (1–2, 3–6, and 7–12 years) and allocated 2:1 by block randomisation to receive 9.0×10^5 *Pf*SPZ Vaccine or placebo (normal saline), administered by direct venous inoculation on days 1, 8, and 29. Artemether–lumefantrine was given before the third vaccination to clear latent parasitaemia. The co-primary endpoints were safety, evaluated in the intention-to-treat population by severe adverse events within 7 days (solicited) and 28 days (unsolicited) of vaccination and by serious adverse events; and vaccine efficacy, measured as time to first *P falciparum*-positive thick blood smear (TBS), 2–26 weeks after immunisation in those who received three vaccinations (ie, the modified intention-to-treat population). The trial was registered at ClinicalTrials.gov, NCT03521973, and is complete.

Findings Between June 21, 2018, and April 30, 2019, 345 children were assessed for eligibility, of whom 200 were enrolled to the study: 134 were allocated to receive *Pf*SPZ Vaccine and 66 to receive placebo. 192 participants received three vaccinations and comprised the modified intention-to-treat population. Systemic adverse events were reported by 33 (25%) of 134 participants in the vaccine group (47 events) and 15 (23%) of 66 participants in the placebo group (25 events); subjective fever was the most reported event in both groups. Three grade 3 systemic adverse events were reported (two cases of elevated body temperature and one case of subjective fever),

all in the placebo group. 32 serious adverse events were reported across 22 study participants, 13 (10%) of 134 in the vaccine group and nine (14%) of 66 in the placebo group, all of which were considered unrelated to the intervention. There were no treatment-related deaths. 25 (19%) of 129 vaccine recipients and 14 (23%) of 63 placebo recipients became TBS-positive for *P falciparum* at 2–26 weeks after vaccination. The age-stratum-adjusted vaccine efficacy (1–hazard ratio) was 9% (95% CI −75 to 53; p=0.78).

Interpretation *Pf* SPZ Vaccine is well tolerated and safe, but it did not prevent *P falciparum* infection in children in Gabon. Whether presumptive treatment during immunisation or more potent *Pf* SPZ vaccines can establish vaccine efficacy is currently under investigation.

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Research in context

Evidence before this study

Immunisation with radiation-attenuated *Plasmodium falciparum* sporozoite (*Pf*SPZ) Vaccine (Sanaria, Rockville, MD, USA), administered by direct venous inoculation, has been extensively evaluated in adults living in Europe, the USA, and Africa. The vaccine is safe and well tolerated and, in controlled human malaria infection experiments, has shown up to 100% vaccine efficacy against homologous (vaccine strain) infection lasting at least 13–14 months and 80% vaccine efficacy against heterologous (non-vaccine strain) infection lasting at least 8 months. In African adults exposed to natural P falciparum infection and disease, *Pf*SPZ Vaccine-induced efficacy is about 60% and lasts at least 18–21 months without boosting. This protection against natural infection in adults has been demonstrated only when presumptive treatment has been administered before the first vaccine dose to clear latent parasitaemia. Vaccination regimens tested have included three doses or more given 8 weeks apart or a shortened regimen of three doses administered on days 1, 8, and 29. Protection in children has not yet been investigated.
We searched PubMed for clinical trials published from database inception to May 25, 2024, with no language restrictions, using the terms "(*Pf*SPZ vaccine) and (efficacy) and (children)". We found four published studies: one, conducted in Kenya, assessed the safety, tolerability, immunogenicity, and efficacy of *Pf*SPZ Vaccine, whereas three, conducted in Kenya, Tanzania, and Equatorial Guinea, assessed the safety, tolerability, and immunogenicity of the vaccine without an efficacy component. The latter three trials showed *Pf*SPZ Vaccine to be safe, well tolerated, and immunogenic at all doses—including the highest dose studied, 1.8×10^6 *Pf*SPZ. The efficacy study in Kenya was a randomised, double-blind, placebo-controlled trial (using normal saline as placebo) in 336 infants aged 5–12 months. The primary protection endpoint was efficacy against *P falciparum* infection in infants in a region of high transmission in western Kenya. Infants received 4.5×10^5, 9.0×10^5, or 1.8×10^6 *Pf*SPZ or saline at 0, 8, and 16 weeks and were followed for 12 months. The infants did not receive presumptive treatment before immunisation, although they received antimalarials before the third immunisation to enable surveillance for new infections during the follow-up period. Although the vaccine was safe and well tolerated, no measurable protective efficacy was observed compared with controls at the primary 6-month timepoint. The vaccine efficacy was 0.8% (95% CI −26.8 to 22.4; p=0.95)

in children who received 4.5×10^5 *Pf*SPZ, −6.5% (−34.7 to 15.8; p=0.60) in children who received 9.0×10^5 *Pf*SPZ, and 12% (−14.6 to 32.5; p=0.34) in children who received 1.8×10^6 *Pf*SPZ. In a post-hoc subgroup analysis of infants receiving 1.8×10^6 *Pf*SPZ, protection against clinical malaria was 46% (p=0.027) during the first 3 months of follow-up. No cellular immune responses to the vaccine were detected in these children. It was hypothesised that the reduced capacity of the infant immune system to develop potent T-cell responses, the lack of presumptive treatment before the first dose of vaccine, or both were responsible for the low efficacy compared with adults.

Added value of this study

We found that three doses of 9.0×10^5 *Pf*SPZ Vaccine administered on days 1, 8, and 29 were safe, well tolerated, and immunogenic in children in Gabon, but did not protect against *P falciparum* infection during the 6-month follow-up period. This finding suggests broadening the clinical development of the *Pf*SPZ Vaccine towards combinations of interventions, including presumptive treatment, to induce protective efficacy.

Implications of all the available evidence

A shortened regimen of *Pf*SPZ Vaccine was safe, but did not prevent new infections in children aged 1–12 years. Data obtained since the completion of this trial showed that presumptive use of antimalarial drugs before immunisation was required for *Pf*SPZ Vaccine to be protective in malaria-endemic settings. This approach will be tested in children, and the systematic development of optimised, combined interventions for all vulnerable groups is a research priority. Cumulative evidence of the safety of *Pf*SPZ Vaccine across age groups and regimens and durable efficacy in adults supports the current efforts to develop more potent second-generation and third-generation *Pf*SPZ vaccines.

Introduction

The potential health and economic benefits of eradicating malaria, which accounted for 597 000 deaths in 2023,[104] are large. Within the next 20 years, malaria elimination could save about 11 million lives, averting 4 billion cases of malaria and preventing economic losses of up to US$2 trillion.[105,106] Sub-Saharan Africa would benefit most from these gains, accounting for 82% of saved lives, 80% of cases averted, and 65% economic gains. A safe and highly efficacious malaria vaccine is needed to complement insecticide-treated bed nets, early diagnosis, prompt treatment with effective therapies, and intermittent preventive strategies—such as seasonal malaria chemoprophylaxis—if malaria eradication is to be achieved.[107,108] Pre-erythrocytic vaccines, which target sporozoites and infected hepatocytes, can induce sterile immunity, as shown in the context of controlled human malaria infections with sporozoites, and have protected a considerable proportion of primarily adult participants after exposure to natural infections.[109–111] RTS,S/AS01 and R21/Matrix-M are the first malaria vaccines recommended by WHO for young children living in areas with moderate-to-high malaria transmission. However, the protection induced by these first-generation vaccines is suboptimal[112] and, in addition to providing only partial efficacy, the need for repeated boosters might reduce their public health impact. Attenuated *Plasmodium falciparum* sporozoite (*Pf*SPZ) vaccines aim to induce more robust (ie, stronger, broader, and longer-lasting) protection.

*Pf*SPZ Vaccine (Sanaria, Rockville, MD, USA) is a pre-erythrocytic vaccine that is administered by direct venous inoculation and consists of aseptic, purified, live (ie, metabolically active), cryopreserved, radiation-attenuated *Pf*SPZ, produced in compliance with good manufacturing practices and meeting all regulatory requirements. Irradiated *Pf*SPZ arrest development early during the liver stage and induce potent pre-erythrocytic immunity. Data from malaria-endemic countries show that *Pf*SPZ Vaccine is safe and well tolerated in infants, children, and adults (age range 5 months–61 years),[113–116] and the vaccine is being developed to prevent *P falciparum* infection, disease, and transmission in travellers and populations living in malaria-endemic settings, especially pregnant women and children in Africa.[117] Protective efficacy of up to 100% has been shown in controlled human malaria infection trials.[118,119] In randomised, placebo-controlled clinical trials in malaria-endemic countries, vaccine efficacy of about 60% against naturally occurring *P falciparum* infection has been reported.[120–1123] Over the past 7 years, a condensed

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111 — Diawara H, Healy SA, Mwakingwe-Omari A, et al. Safety and efficacy of PfSPZ Vaccine against malaria in healthy adults and women anticipating pregnancy in Mali: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. Lancet Infect Dis 2024; 24: 1366–82.

112 — Asante KP, Mathanga DP, Milligan P, et al. Feasibility, safety, and impact of the RTS,S/AS01E malaria vaccine when implemented through national immunisation programmes: evaluation of cluster-randomised introduction of the vaccine in Ghana, Kenya, and Malawi. Lancet 2024; 403: 1660–70.

113 — Jongo SA, Church LWP, Mtoro AT, et al. Safety and differential antibody and T-cell responses to the Plasmodium falciparum sporozoite malaria vaccine, PfSPZ Vaccine, by age in Tanzanian adults, adolescents, children, and infants. Am J Trop Med Hyg 2019; 100: 1433–44.

immunisation regimen consisting of three doses of 9.0×10^5 *Pf*SPZ Vaccine at 1, 8, and 29 days has been developed, and has shown high levels of protection against controlled human malaria infection with both homologous parasites (the same parasite strain as in the vaccine) and heterologous parasites (a genetically different clone of *P falciparum*)[124] in malaria-naive adult vaccine recipients.[125]

An age de-escalation trial in adults and children in Tanzania found that *Pf*SPZ Vaccine induced relatively greater immunogenicity in younger children (aged 6–10 years) than in other age groups, suggesting that cumulative previous exposure to malaria infections could reduce the response to vaccination.[126] Nevertheless, *Pf*SPZ Vaccine administered at doses of 4.5×10^5, 9.0×10^5, and 1.8×10^6 *Pf*SPZ to infants in Kenya did not provide protection against naturally acquired *P falciparum* infection. Post-hoc analysis suggested that 1.8×10^6 *Pf*SPZ provides modest protection at 3 months post immunisation.[127] It was hypothesised that an age-dependent dysregulation of γδ T cells that affects cellular memory responses against malaria parasites[128] was responsible for the lack of protection observed. Since 2020, evidence has accumulated that the immunomodulatory effect of low-level blood-stage infections could contribute to suppressed vaccine immunogenicity.[129,130]

Children from the age of 1–12 years not only bear a substantial burden of disease in highly malaria-endemic areas, but also represent a potential immunological Goldilocks age (ie, they are old enough to develop strong cellular immunity and young enough that previous exposure has not yet compromised the immunogenicity of malaria antigens), and form the greatest reservoir for onward transmission.[131] Targeting vaccination towards this age group could therefore be particularly efficacious, both at an individual level and to achieve a substantial reduction in malaria transmission in sub-Saharan Africa.[132,133]

Here we report the first results of a randomised, double-blind, placebo-controlled trial designed to assess the safety and efficacy of the *Pf*SPZ Vaccine in children aged 1–12 years living in and around Lambaréné, Gabon—an area with low-to-moderate (0.2–1 episode per person-year) perennial malaria transmission.[134–136]

114 — Steinhardt LC, Richie TL, Yego R, et al. Safety, tolerability, and immunogenicity of Plasmodium falciparum sporozoite vaccine administered by direct venous inoculation to infants and young children: findings from an age de-escalation, dose-escalation, double-blind, randomized controlled study in western Kenya. Clin Infect Dis 2020; 71: 1063–71.

115 — Jongo SA, Urbano Nsue Ndong Nchama V, Church LWP, et al. Safety and immunogenicity of radiation-attenuated PfSPZ Vaccine in Equatoguinean infants, children, and adults. Am J Trop Med Hyg 2023; 109: 138–46.

116 — Oneko M, Steinhardt LC, Yego R, et al. Safety, immunogenicity and efficacy of PfSPZ Vaccine against malaria in infants in western Kenya: a double-blind, randomized, placebo-controlled phase 2 trial. Nat Med 2021; 27: 1636–45.

117 — Idem 110.

118 — Sissoko MS, Healy SA, Katile A, et al. Safety and efficacy of a three-dose regimen of Plasmodium falciparum sporozoite vaccine in adults during an intense malaria transmission season in Mali: a randomised, controlled phase 1 trial. Lancet Infect Dis 2022; 22: 377–89.

119 — Seder RA, Chang LJ, Enama ME, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science 2013; 341: 1359–65.

120 — Idem 111.

121 — Idem 118.

122 — Sirima SB, Ouédraogo A, Tiono AB, et al. A randomized controlled trial showing safety and efficacy of a whole sporozoite vaccine against endemic malaria. Sci Transl Med 2022; 14: eabj3776.

123 — Sissoko MS, Healy SA, Katile A, et al. Safety and efficacy of PfSPZ Vaccine against Plasmodium falciparum via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial. Lancet Infect Dis 2017; 17: 498–509.

124 — Moser KA, Drábek EF, Dwivedi A, et al. Strains used in whole organism Plasmodium falciparum vaccine trials differ in genome structure, sequence, and immunogenic potential. Genome Med 2020; 12: 6.

125 — Mordmüller B, Sulyok Z, Sulyok M, et al. A PfSPZ Vaccine immunization regimen equally protective against homologous and heterologous controlled human malaria infection. NPJ Vaccines 2022; 7: 100.

Methods

Study design and participants

This randomised, double-blind, placebo-controlled, phase 2 trial was conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL), in Lambaréné, Gabon. Healthy participants aged 1–12 years who resided in the study area were recruited from the local community by a field worker who provided information about the trial to interested families. Participants were recruited into three age strata: 7–12 years, 3–6 years, and 1–2 years. Children whose potential participation in the study was considered by their legal guardians were scheduled for an information visit at CERMEL; at this visit, the study aims and procedures and the informed consent process were discussed with the child's legal guardian. Written informed consent was obtained from each legal guardian before all study procedures.

Key exclusion criteria included previous receipt of any malaria vaccine and acute or severe significant disease. A complete list of inclusion and exclusion criteria is available in the thesis supplementary.

The trial was conducted in accordance with international guidelines and Gabonese regulations. The trial dossier was reviewed by the Scientific Committee of CERMEL (SRC2016-06) and approved by the Institutional Ethics Committee of CERMEL (CEI008/2018), the National Ethics Committee of Gabon (004/2018/PR/SG/CNE), the National Drug Regulatory Authority of Gabon (ATU/0019/18), and the US Food and Drug Administration. The trial is registered at ClinicalTrials.gov, NCT03521973, and is complete.

Randomisation and masking

Participants were randomly assigned in a 2:1 ratio to receive *Pf*SPZ Vaccine or placebo. The randomisation sequence was generated by the Mersenne Twister random number generator[137] implemented in R. Randomisation was age-stratified (7–12 years, 3–6 years, and 1–2 years [12–35 months]) and in randomly permuted blocks of size 3, 6, or 9. An unmasked pharmacist, who was not involved in participant management or diagnostic activities, kept sealed envelopes containing the allocation of individual participants. The envelope was opened immediately before the vaccine vial for the first injection was thawed, diluted, and loaded into a 1 mL syringe with a fixed 25-gauge needle. Normal saline was used as placebo. Both products are visually

126 – Idem 113.

127 – Idem 116.

128 – Zaidi I, Diallo H, Conteh S, et al. γδ T cells are required for the induction of sterile immunity during irradiated sporozoite vaccinations. J Immunol 2017; 199: 3781–88.

129 – Idem 111.

130 – Murphy SC, Deye GA, Sim BKL, et al. PfSPZ-CVac efficacy against malaria increases from 0% to 75% when administered in the absence of erythrocyte stage parasitemia: a randomized, placebo-controlled trial with controlled human malaria infection. PLoS Pathog 2021; 17: e1009594.

131 – Drakeley C, Abdulla S, Agnandji ST, et al. Longitudinal estimation of Plasmodium falciparum prevalence in relation to malaria prevention measures in six sub-Saharan African countries. Malar J 2017; 16: 433.

132 – Idem 113.

133 – Idem 116.

134 – Idem 131.

135 – RTS,S Clinical Trials Partnership. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. PLoS Med 2014; 11: e1001685.

136 – Asante KP, Abdulla S, Agnandji S, et al. Safety and efficacy of the RTS,S/AS01E candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. Lancet Infect Dis 2011; 11: 741–49.

137 – Matsumoto M, Nishimura T. Mersenne twister: a 623-dimensionally equidistributed uniform pseudo-random number generator. ACM Trans Model Comput Simul 1998; 8: 3-30. 1

38 – Idem 110.

indistinguishable and have a similar viscosity (ie, there is no notable difference while injecting). The same syringe type was used for both study products. The pharmacist prepared the study product and passed syringes labelled with the participant identification code to masked clinical team members for vaccination by direct venous inoculation. All other individuals involved in the trial—participants, those assessing outcomes, and those analysing data—were masked to group assignment.

Procedures

At each child's screening visit, a medical history was taken, a physical examination was conducted, and blood was collected for haematological, biochemical, and serological investigations. Data on age, sex, and residence of the participants were collected from medical records. Potential participants who had a positive thick blood smear (TBS) for *Plasmodium* spp but were otherwise eligible for participation received a weight-adjusted 3-day course of artemether–lumefantrine and were rescheduled for subsequent study procedures according to the medical judgement of the study investigators. For each eligible participant, a prevaccination visit was scheduled no more than 8 weeks after screening, at which the assessment of inclusion and exclusion criteria was repeated. *Pf* SPZ Vaccine (Sanaria, Rockville, MD, USA)[138] and normal saline were administered by direct venous inoculation in 0.5 mL on days 1, 8, and 29. *Pf* SPZ Vaccine was given at a dose of 9.0×10^5 *Pf* SPZ within 30 min of thawing.

Solicited local and systemic adverse events were monitored for 7 days after each vaccination, unsolicited adverse reactions for 28 days after each vaccination, and serious adverse events from the first vaccination to the final visit at week 104 (±2) after the third vaccination. Haematological and clinical chemistry investigations were scheduled at screening; before each vaccination; and at months 2, 6, 12, and 24.

2 weeks before the third vaccination, all participants received a presumptive, weight-adjusted, 3-day course of oral artemether–lumefantrine in the form of dispersible tablets to clear any latent parasitaemia. Intake was directly observed by study staff. After the third vaccination, parasitaemia was monitored by TBS every 2 weeks until 26 weeks after the third vaccination and thereafter every 4 weeks. In addition, children were monitored at home by their guardians for potential adverse events and symptoms suggestive of malaria (eg, fever); in the case of symptoms, they were advised to attend

an outpatient visit. Quantitative PCR (qPCR) was conducted retrospectively on screening and prevaccination blood samples.

Anti-*P falciparum* circumsporozoite protein (*Pf*CSP) and exported protein 1 (EXP1) IgM and IgG antibodies were assessed by a standardised ELISA before the first vaccination and 2 weeks after the third vaccination, using a net optical density (OD) of 1.0 as a threshold for antibody positivity.[139,140]

Outcomes

The primary safety endpoint was the occurrence and frequency of grade 3 solicited adverse events within 7 days of each vaccination, grade 3 unsolicited adverse events from the time of first vaccination until 28 days after the third vaccination, and serious adverse events from the time of first vaccination until the end of the study. Unsolicited adverse events were coded using the Medical Dictionary for Regulatory Activities version 23.0 and analysed using preferred terms and system organ class categories. The safety population comprised all participants who received at least one vaccination with *Pf*SPZ Vaccine or placebo, regardless of protocol compliance, and was the same as the intention-to-treat population.

The primary efficacy endpoint was vaccine efficacy, measured as time to first episode of naturally acquired *P falciparum* infection in vaccine recipients versus placebo recipients in the modified intention-to-treat population, from 2 weeks to 26 weeks after the third vaccination. The modified intention-to-treat populations consisted of all children who received three doses of *Pf*SPZ Vaccine or placebo, regardless of schedule. Active case detection took place every 2 weeks and was complemented by passive case detection in participants presenting with symptoms suggestive of malaria, as reported by the children's parents or guardians. We defined *P falciparum* infection as the detection of at least two *P falciparum* parasites in a TBS by microscopic examination of 0.5 μL of blood by two independent readers, regardless of the presence or absence of malaria symptoms. To assess immunogenicity, humoral immune responses against *Pf*CSP were measured before the first vaccination and 2 weeks after the third vaccination as exploratory immunological endpoints. We characterised differences in immune responses between the *Pf*SPZ Vaccine and placebo groups, as well as between vaccine recipients who became TBS-positive during follow-up and those in whom no *P falciparum* infection was detected.

139 – Idem 113.

140 – Mordmüller B, Surat G, Lagler H, et al. Sterile protection against human malaria by chemoattenuated PfSPZ Vaccine. Nature 2017; 542: 445–49.

141 – Idem 123.

142 – Sagara I, Giorgi R, Doumbo OK, Piarroux R, Gaudart J. Modelling recurrent events: comparison of statistical models with continuous and discontinuous risk intervals on recurrent malaria episodes data. Malar J 2014; 13: 293.

Statistical analysis

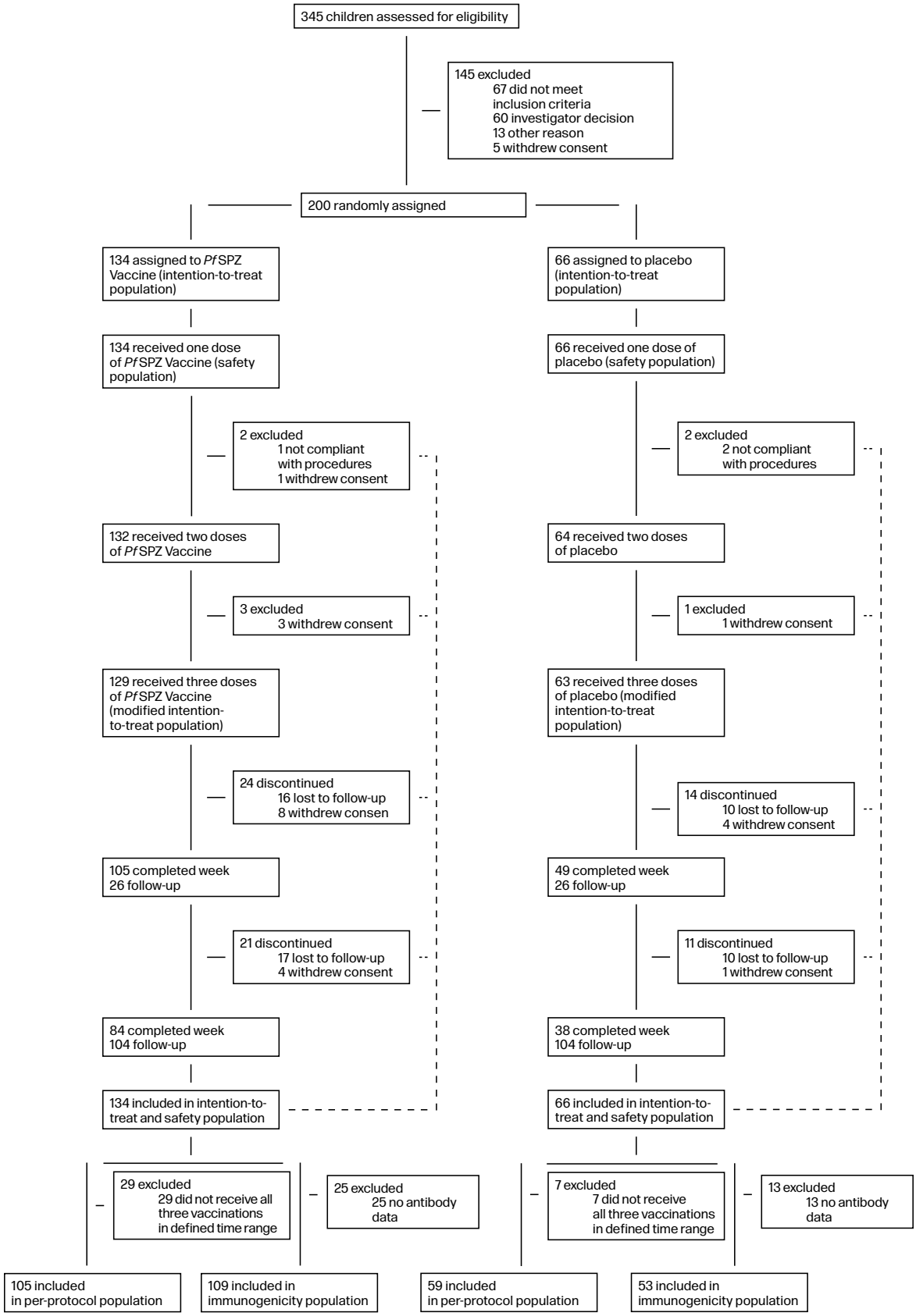
Sample size calculations were based on assumptions about the efficacy of *Pf* SPZ Vaccine from previous studies.[141] Assuming an incidence of 0.5 episodes of *P falciparum* parasitaemia per person-year in the placebo group, the study was powered to detect a hazard ratio (HR) of at least 0.375 (62.5% protective efficacy) with 80% power and 2.5% one-sided type-1 error. The sample size was calculated using R with the package gsDesign using the command nSurvival. All statistical analyses were conducted in R version 4.1.3.

We measured the HR between the time to event of the first *P falciparum* parasitaemia in the *Pf* SPZ Vaccine group versus placebo group in the modified intention-to-treat population, with vaccine efficacy defined as 1 – HR. Vaccine efficacy was quantified using a Cox proportional hazards model with allocation and age stratum as variables. We also conducted the primary efficacy analysis in the per-protocol population, which was defined as all participants who received three vaccinations within the prespecified time windows (±1 day for the second vaccination and ±3 days for the third vaccination) and were followed until the first malaria episode or for at least 26 weeks after the last vaccination.

A predetermined secondary efficacy analysis of *Pf* SPZ Vaccine efficacy against clinical malaria, defined as the first episode of *P falciparum* parasitaemia of any density associated with a temperature of greater than 38°C or a history of fever within the past 24 h, was planned in a hierarchical testing approach—ie, conditional on a significant outcome of the primary efficacy analysis.

Exploratory efficacy analyses were vaccine efficacy at 104 weeks, and assessment of a possible association between recent (ie, within the previous 14 days) or current *P falciparum* infection at the time of first vaccination and protective efficacy, by including prevaccination TBS positivity, PCR positivity, and immunological variables as predictors in a multivariate Cox proportional hazards model. Cox models with Andersen–Gill extension were used to model recurring events of *P falciparum* parasitaemia.[142] The trial was overseen by a data and safety monitoring board.

Figure 8: Trial profile
Pf SPZ=*Plasmodium falciparum* sporozoite.



Role of the funding source

The German Center for Infection Research funded the trial conduct, the European Developing Countries Clinical Trials Partnership funded the research team, and the National Institute of Allergy and Infectious Diseases (National Institutes of Health) and the National Institutes of Health funded the vaccine manufacture. These funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between June 21, 2018, and April 30, 2019, 345 children were screened for eligibility, of whom 200 were enrolled in the study. Of these 200 participants, 134 were randomly allocated (stratified by age: 1–2 years, 3–6 years, and 7–12 years) to receive *Pf* SPZ Vaccine and 66 to receive placebo (figure 8). All 200 participants received at least their first vaccination and comprised the safety population. 164 participants (105 in the vaccine group and 59 in the placebo group) received all three vaccinations within the prespecified time windows and were followed up for at least 26 weeks after their third vaccination, comprising the per-protocol population. Baseline characteristics of the enrolled participants were balanced between groups and are shown in the table 6.

70 (35%) of 200 participants had evidence of recent (ie, within the previous 14 days) blood-stage *P falciparum* infection before commencing vaccinations; 17 (24%) of these 70 participants were TBS-positive at the screening and/or the prevaccination visit and were treated with a standard paediatric course of artemether–lumefantrine before reassessment and enrolment a few weeks later. 68 participants tested positive by qPCR, which was conducted retrospectively on screening and prevaccination blood samples, 53 of which were positive. Two samples that were TBS-positive were PCR-negative, probably due to a technical or logistical issue.

Local adverse events (pain, tenderness, and/or pruritus) were reported by three (2%) of 134 participants in the vaccine group and four (6%) of 66 participants in the placebo group (figure 9, thesis supplementary). No participant had more than one local adverse event. Except for one case of grade 2 pruritus in the placebo group, all local adverse events were grade 1.

33 (25%) of 134 participants in the vaccine group and 15 (23%) of 66 participants in the placebo group reported systemic adverse events (figure 9, thesis supplementary). 47 systemic adverse events were reported in the vaccine group and 25 in the placebo group. Subjective fever was the most reported systemic adverse event in both groups. Three grade 3 systemic adverse events were reported (two cases of elevated body temperature and one case of subjective fever), all in the placebo group. All other systemic adverse events were grade 1 or grade 2.

Unsolicited adverse events occurred in 104 (78%) of 134 participants in the vaccine group and 46 (70%) of 66 participants in the placebo group. 356 unsolicited adverse events occurred in the vaccine group and 161 in the placebo group. One unsolicited adverse event in the vaccine group and three in the placebo group were grade 3; all were considered unrelated to *Pf* SPZ Vaccine or placebo. Of the unsolicited adverse events, 217 (61%) of 356 in the vaccine group and 71 (44%) of 161 in the placebo group were considered to be at least possibly related to the intervention. Relatively high numbers of possibly related adverse events were observed in the system organ class of respiratory, thoracic, and mediastinal disorders in the vaccine group (n=71; 32% of all possibly related adverse events) compared with the placebo group (n=15; 21% of all possibly related adverse events). Within this system organ class category, the occurrences of cough (n=44 vs n=10) and rhinorrhoea (n=11 vs n=1) were higher in the vaccine group than in the placebo group (appendix pp 5–13). No pattern related to vaccination sequence (first, second, or third dose; χ^2; p=0.67) or co-occurrence of other adverse events was evident. Allocation to the vaccine group was not significantly associated with the occurrence of a possibly related unsolicited adverse event in the system organ class of respiratory, thoracic, and mediastinal disorders in a logistic regression model (p=0.30).

Leukocytosis occurred within 7 days after vaccination in three (5%) of 66 participants in the placebo group and 21 (16%) of 134 participants in the vaccine group (p=0.022, Fisher's exact test; thesis supplementary, with leukocyte concentrations ranging from 10.3×10^9 to 30.0×10^9 per L. In ten (42%) of these 24 participants, leukocytosis occurred after more than one vaccination. Leukocytosis did not cluster with the vaccination sequence (15 times after dose 1, 12 times after dose 2, and 12 times after dose 3). In all 24 participants, leukocytosis resolved either by the blood draw at the next visit or within 7 days.

Grade 3 or 4 thrombocytopenia was detected at 1 day post-vaccination in four (3%) of 134 participants in the vaccine group. All thrombocyte counts returned to the normal

range in subsequent samples, and all four participants were clinically well.

32 serious adverse events were reported across 22 study participants: 13 (10%) of 134 in the vaccine group and nine (14%) of 66 in the placebo group. All such events were considered unrelated to *Pf*SPZ Vaccine or placebo (thesis supplementary), and were defined as serious because of a requirement for hospitalisation; in 73% (16 of 22) participants, clinical malaria was the most common event.

Time to first *Pfalciparum* infection was analysed 2–26 weeks after the third vaccination in the modified intention-to-treat population as a primary endpoint. 14 (22%) of 63 placebo recipients and 25 (19%) of 129 vaccine recipients became TBS-positive during this period (figure 10); only one of these participants, a vaccine recipient, was in the 1–2 years age group (thesis supplementary),. Vaccine efficacy using a Cox HR model adjusted for age stratum was 9% (95% CI −75 to 53; p=0.78). By the end of the study period (104 weeks), 31 (49%) of 63 placebo recipients and 56 (43%) of 129 vaccine recipients had become *Pfalciparum*-positive (vaccine efficacy 19% [−26 to 47]; p=0.35).

Table 6: Baseline characteristics of the safety population

Characteristic	1–2 years		3–6 years		7–12 years	
	Placebo (n=12)	Vaccine (n=25)	Placebo (n=33)	Vaccine (n=66)	Placebo (n=21)	Vaccine (n=43)
Age at screening, years	2.43 (1.67–2.98)	2.01 (1.01–2.89)	5.38 (2.24–6.76)	5.10 (3.00–6.96)	9.63 (7.03–11.8)	9.50 (7.04–12.6)
Sex						
Male	8 (67%)	10 (40%)	17 (52%)	36 (55%)	9 (43%)	19 (44%)
Female	4 (33%)	15 (60%)	16 (48%)	30 (45%)	12 (57%)	24 (56%
Weight, kg	12.4 (8.0–15.0)	12.0 (8.0–16.0)	18.0 (11.0–22.0)	16.0 (10.0–26.0)	28.0 (18.5–40.0)	26.0 (18.0–44.1)
Height, cm	87.5 (76.0–90.0)	83.5 (72.0–94.0)	110.0 (85.0 –123.0)	107.0 (85.0 –125.0)	133.0 (118.0 –156.0)	132.0 (105.0 –155.0)

Data are median (IQR) or n (%).
*One participant, who was younger than 36 months at the time of randomisation, was misallocated to the age 3–6 years stratum. To adhere to the prespecified allocation ratios, it was decided not to correct this misallocation for analyses.

Figure 9: Solicited local and systemic adverse events
Solicited adverse events were recorded from each inoculation until 7 days thereafter. Data show the cumulative occurrence of adverse events per participant per dose. The green bars separate local (above) from systemic (below) adverse events. Adverse events that occurred at more than one severity grade in a given participant per dose are shown only at the highest respective grade

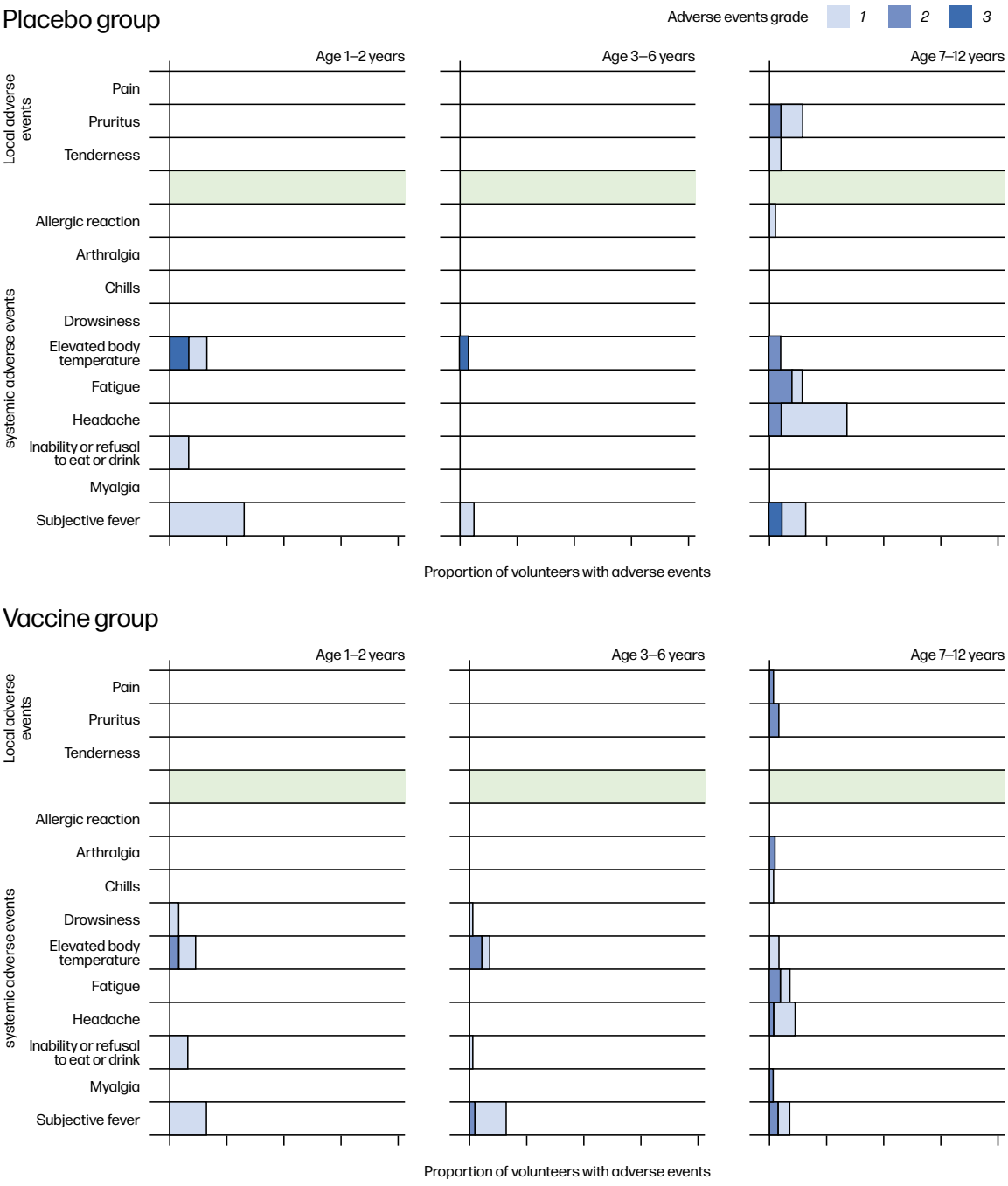
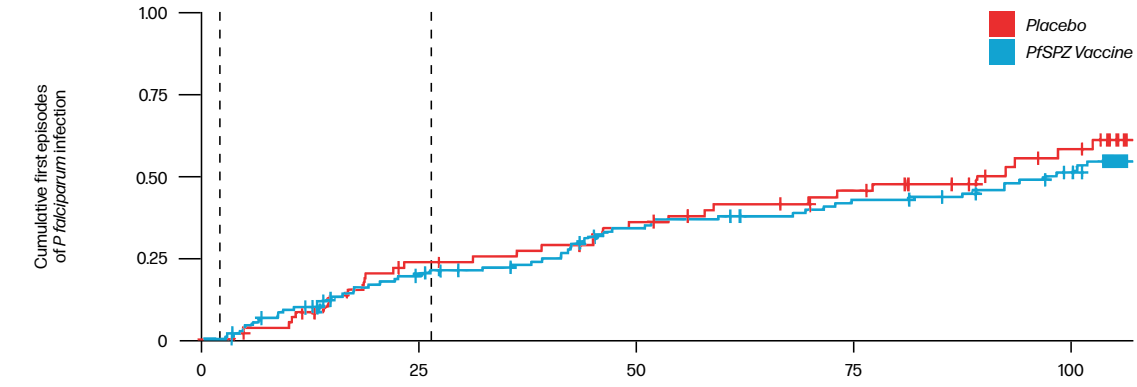


Figure 10: Kaplan–Meier estimates of the time to first or only episode of *Plasmodium falciparum* infection per participant
Between week 1 and week 26 after the third vaccination (enclosed within the dotted lines), 38 episodes of *P falciparum* malaria (>35 episodes, as prespecified by the protocol to meet 80% power and 2.5% one-sided type 1 error) were cumulated to determine the efficacy primary endpoint. *Pf* SPZ=*Plasmodium falciparum* sporozoite.



Number at risk (number of events)					
Placebo	62 (0)	44 (14)	36 (21)	27 (26)	14 (31)
Pf SPZ Vaccine	125 (0)	91 (24)	70 (39)	58 (48)	43 (56)

In the 14 days before the first immunisation, four (2%) of 200 participants were TBS-positive and subsequently received antimalarial treatment. All were vaccine recipients and did not test positive for *P falciparum* throughout the primary follow-up period.

53 participants tested PCR-positive in the 14 days before the first immunisation, of whom 12 received placebo and 41 received the vaccine. The proportion of PCR-positive participants was higher in the older stratum (7–12 years; 24 [39%] of 61 participants) than in the middle (3–6 years; 22 [22%] of 98 participants) and younger (1–2 years; seven [21%] of 33 participants) strata. Of these 53 participants, four (33%) of 12 participants who received placebo and five (12%) of 41 participants who received the vaccine developed *P falciparum* parasitaemia during the primary follow-up period (p=0.18, Fisher's exact test).

All antibody assay results are provided in the thesis supplementary,. 101 (93%) of 109 vaccine recipients seroconverted for anti-*Pf*CSP IgG antibodies, compared with five (9%) of 53 placebo recipients (Wilcoxon– Mann–Whitney test, p<0.0001). The net OD 1.0 anti-*Pf*CSP IgG level at 2 weeks after the third vaccination was approximately 3000 times higher

in the vaccine group than in the placebo group (p<0.0001, Wilcoxon rank sum test; figure 11A), irrespective of the age stratum. Within the vaccine group, no significant difference was observed in the net OD 1.0 anti-*Pf*CSP IgG level, 2 weeks after the final vaccination, between those who tested positive for *Pfalciparum* parasitaemia and those who remained uninfected during the primary follow-up period of 2–26 weeks after the third vaccination (p=0.90, figure 11A, B). Net OD 1.0 anti-*Pf*CSP IgM levels also did not differ significantly between uninfected and infected vaccine recipients (p=0.17, figure 11C, D). A trend towards decreasing net vaccine-induced immune response was seen with increasing age strata of vaccinees (thesis supplementary), but age was not significantly associated with post-vaccination anti-*Pf*CSP IgG titres (p=0.57).

Higher levels of preimmunisation anti-*Pf*CSP IgG (a binary predictor, OD 1.0 of >100) were significantly associated with recurrent events of *Pfalciparum* parasitaemia, using a Cox model with Andersen–Gill extension (HR 1.65 [95% CI 1.12–2.43], p=0.011). Vaccine recipients without recent *P falciparum* parasitaemia had 2.7 times higher anti-*Pf*CSP IgG levels after vaccination than those with recent *Pfalciparum* parasitaemia (defined as PCR-positivity within 14 days before the first immunisation; p=0.0004 using a linear regression model with log10 of post-vaccination *Pf*CSP IgG level as the dependent variable and log10 of baseline *Pf*CSP IgG levels and PCR-positivity as independent variables).

Within the vaccine group, the pre-immunisation median OD 1.0 anti-*Pf*CSP IgG levels were higher in participants who became infected during follow-up (n=62) than in those who remained uninfected (n=33), but the difference was not significant (p=0.073; figure 12A). A similar non-significant relationship was seen for pre-immunisation median OD 1.0 anti-*Pf*EXP1 IgG levels (n=154 vs n=72; figure 12B).

For all participants—both vaccine recipients and placebo recipients—the median OD 1.0 anti-*Pf*CSP IgG level before immunisation among those who became infected during follow-up (n=54) was significantly higher than among those who remained uninfected (n=30; p=0.039; figure 12C). A similar relationship was seen for median OD 1.0 anti-*Pf*EXP1 IgG levels before immunisation (n=190 vs n=83), but was not significant (p=0.075; figure 12C, D).

Figure 11: Antibody responses to *Pf* CSP

Net OD 1·0 anti-*Pf* CSP IgG (A), ratio of OD 1·0 anti-*Pf* CSP IgG (B), net OD 1·0 anti-*Pf* CSP IgM (C), and ratio of OD 1·0 anti-*Pf* CSP IgM (D) in uninfected and infected participants in the vaccine and placebo groups, 2 weeks after the third dose of either *Pf* SPZ Vaccine or placebo. Participants were categorised as either uninfected (protected) or infected at the primary follow-up period (2–26 weeks after the third inoculation). Filled circles represent uninfected individuals and open circles indicate infected individuals, all of whom received $9{\cdot}0 \times 10^5$ *Pf* SPZ Vaccine or placebo on days 1, 8, and 29. The midline represents the median and error bars are IQR. All vaccinated groups differed significantly from placebo (p<0·0001). Significance was assessed using the Wilcoxon–Mann–Whitney test. OD=optical density. *Pf* CSP=*Plasmodium falciparum* circumsporozoite protein. *Pf* SPZ=*Plasmodium falciparum* sporozoite.

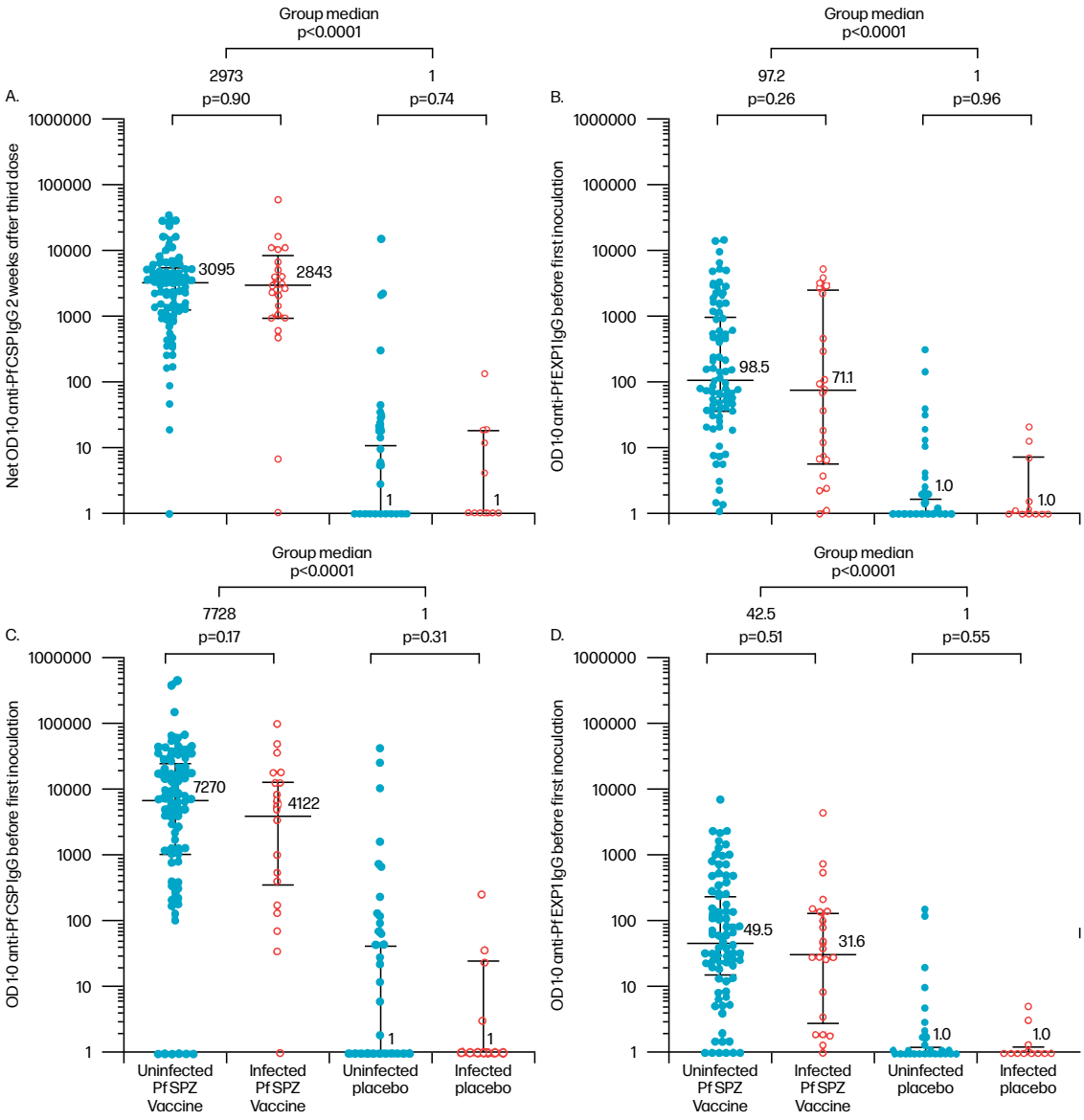
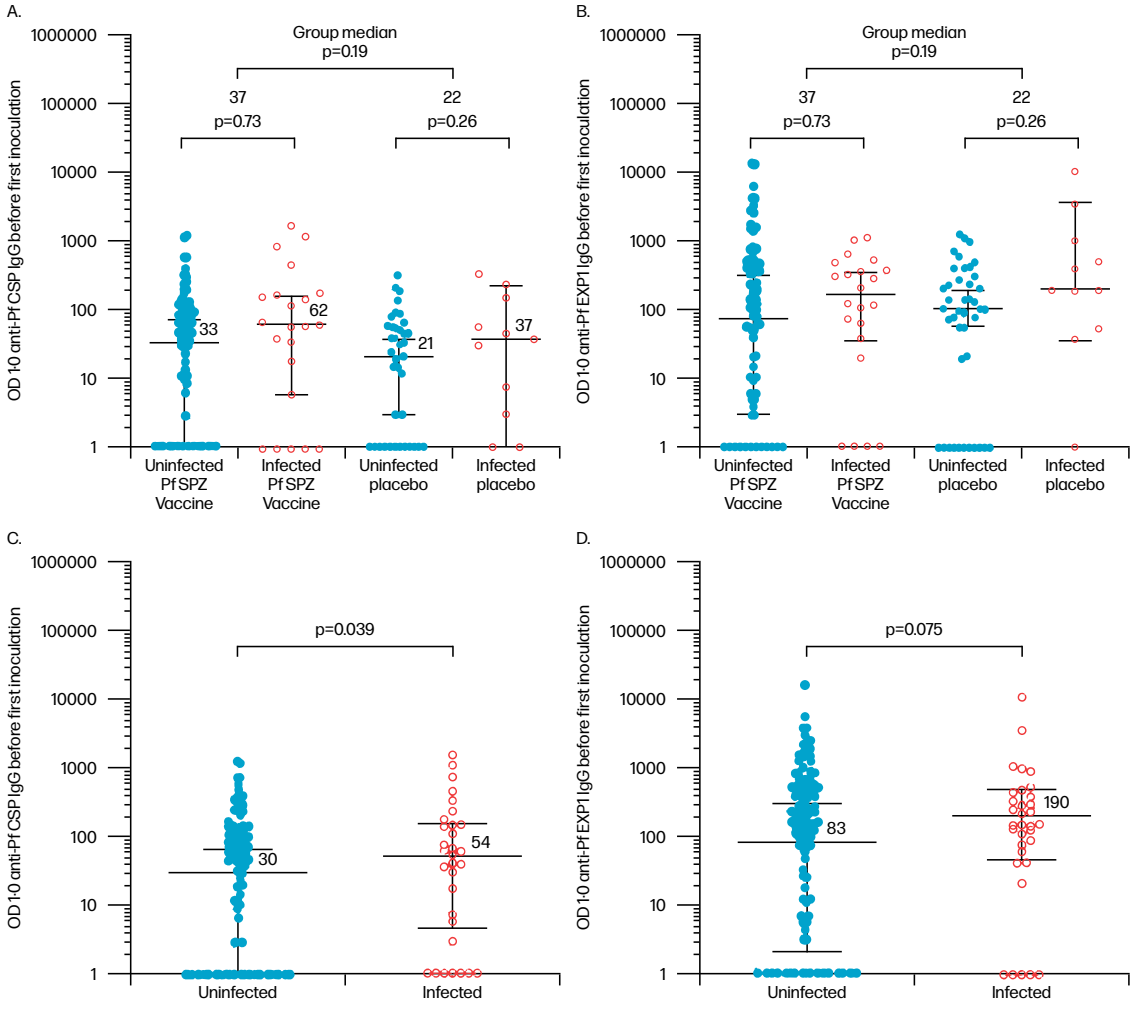


Figure 12: Pre-inoculation antibody levels

OD 1·0 anti-*Pf* CSP IgG (A, C) and OD 1·0 anti-*Pf* EXP1 IgG (B, D) in uninfected and infected participants in the vaccine and placebo groups separately (A, B) and for both groups combined (C, D) before the first inoculation with either *Pf* SPZ Vaccine or placebo. Participants were categorised as either uninfected (protected) or infected at the primary follow-up period (2–26 weeks after the third inoculation). Filled circles represent uninfected individuals and open circles indicate infected individuals, all of whom received 9·0 × 10^5 *Pf* SPZ Vaccine or placebo on days 1, 8, and 29. The midline represents the median and error bars are IQR. Significance was assessed using the Wilcoxon–Mann–Whitney test. OD=optical density. *Pf* CSP=*Plasmodium falciparum* circumsporozoite protein. *Pf* EXP1=*Plasmodium falciparum* exported protein 1. *Pf* SPZ=*Plasmodium falciparum* sporozoite.



Discussion

143 – Idem 113.

144 – Idem 114.

145 – Idem 115.

146 – Idem 116.

147 – Idem 111.

148 – Idem 116.

149 – Idem 130.

This trial, conducted in children aged 1–12 years living in a malaria-endemic area in Lambaréné, Gabon, is—to our knowledge—the first to assess the efficacy of the accelerated immunisation regimen of 9.0×10^5 *Pf*SPZ Vaccine on days 1, 8, and 29 in an African paediatric population.

Local solicited adverse events and grade 3 systemic adverse events were rare. Leukocytosis occurred more in vaccine recipients than in placebo recipients, but was transient and judged as clinically not significant. Thrombocytopenia occurred in a few vaccine recipients, but in all cases was transient and resolved quickly. Although our trial corroborated the good safety profile of *Pf*SPZ Vaccine observed in previous paediatric trials,[143–146] future trials investigating the accelerated immunisation regimen will monitor the observed imbalances in adverse events between vaccine recipients and placebo recipients.

In the current trial, presumptive treatment was not given before the first dose. This same immunisation regimen of *Pf*SPZ Vaccine administered to women in Mali who underwent presumptive treatment to clear parasitaemia before the first dose of vaccine had a vaccine efficacy of 57% against naturally acquired *Pfalciparum* infection during pregnancy for 18–21 months without boosting.[147] Concurrent blood-stage parasitaemia during vaccination at patent or subpatent concentrations can eliminate vaccine efficacy induced by both *Pf*SPZ Vaccine[148] and chemo-attenuated *Pf*SPZ (*Pf*SPZ-CVac).[149] In our study, patent microscopic parasitaemia by TBS was an exclusion criterion for vaccination and was treated, after which potential participants were reassessed for enrolment; however, we did not administer presumptive treatment. TBS has a lower detection limit of 10 000–100 000 parasites per mL, meaning that additional participants could have had patent but submicroscopic parasitaemia at the time of immunisation. We retrospectively assessed submicroscopic parasitaemia in blood samples collected in the 2 weeks before the first vaccination by 18S qPCR, which has a sensitivity of 5 parasites per mL: 53 (27%) of 200 participants tested positive by this method. Pre-immunisation qPCR-positivity was not associated with vaccine efficacy. However, despite the 1000-fold increase in sensitivity provided by qPCR, the technique can fail to identify very-low-density parasitaemia or when parasites are sequestered in the spleen, bone marrow, or other organs. Submicroscopic parasitaemia before first vaccination was significantly associated with decreased *Pf*SPZ Vaccine

immunogenicity, as defined by net anti-*Pf*CSP IgG levels 2 weeks after the third vaccination.

P falciparum parasitaemia modulates the immune system and alters its ability to respond to vaccination, mostly resulting in hyporesponsiveness. The consequences of acute *P falciparum* infection include immunosuppressive mechanisms that impede clearance of parasitaemia and maintain anergy to plasmodial proteins[150] at the time of infection, and decreased serological responses to Salmonella enterica serotype Typhi and meningococcal vaccines up to 1 month after infection.[151] However, how long the negative effect of parasitaemia lasts following treatment is unknown. We therefore assessed serological markers of cumulative and recent exposure before vaccination.

High prevaccination anti-*Pf*CSP IgG levels were associated with a higher risk of parasitaemia independently of vaccination. This finding suggests that children in this study, residing in a moderate-to-high transmission setting and thereby exposed to frequent infectious mosquito bites, developed high prevaccination anti-*Pf*CSP IgG titres and had a higher risk of parasitaemia during follow-up.

Among vaccine recipients, we observed similar trends of higher pre-immunisation median OD 1.0 anti-*Pf*CSP IgG levels and median anti-*Pf*EXP1 IgG levels (a marker of recent exposure) in children who became infected. These trends could result from the same underlying process, but could also indicate that cumulative previous exposure suppresses vaccine immunogenicity.[152]

Overall, vaccine-induced antibody levels were relatively low compared with those in previous studies in the same age groups.[153,154] This finding could indicate that malaria is transmitted at a higher intensity in Lambaréné than in the locations of these previous studies (Bagamoyo, Tanzania, and Malabo, Equatorial Guinea), resulting in more intense immunosuppression.

In previous trials of *Pf*SPZ Vaccine in adults and infants, including the trial in Malian women who became pregnant and had received presumptive treatment before the first dose of *Pf*SPZ Vaccine, a correlation has been observed between anti-*Pf*CSP IgG antibody levels 2 weeks after the third dose of vaccine and protection against *P falciparum* infection during the trial.[155–159] By contrast, in this trial, anti-*Pf*CSP IgG and IgM levels 2 weeks after the third vaccination were not predictive of protection against infection during the primary efficacy evaluation period of 26 weeks. Similar results were found in Malian adults when no pre-treatment was used.[160]

150, 151, 152 — Idem 110.

153 — Idem 113.

154 — Idem 115.

155 — Idem 111.

156 — Idem 116.

157 — Idem 118.

158 — Idem 122.

159 — Idem 123.

160 — Idem 111.

In an age de-escalation trial of *Pf*SPZ Vaccine in adults, children, and infants in Tanzania, net anti-*Pf*CSP IgG responses to immunisation decreased from infancy to adulthood, but induced *Pf*SPZ-specific memory CD4 T-cell responses were highest in children aged 6–10 years.[161] Infants aged 5–12 months in Kenya immunised with *Pf*SPZ Vaccine developed anti-*Pf*CSP antibodies, but did not mount T-cell responses, potentially because they lacked sufficient $V\delta2^{+}V\gamma9^{+}$ T cells that could have a role in the induction of such responses.[162] Of note, these infants, who did not have presumptive treatment before the first immunisation, were not protected against malaria beyond 3 months.[163] On the basis of these data from infants and children in east Africa, we hypothesised that there should be an optimal ratio between immune competency and exposure in children aged 1–12 years. Accordingly, the children enrolled in our trial were considered to represent an immunological Goldilocks age. A *Pf*SPZ Vaccine age de-escalation trial in individuals from Equatorial Guinea, published in 2023, substantiated that anti-*Pf*CSP IgG responses to vaccination decreased from infancy to adulthood, but did not assess cellular responses.[164] In our trial, we observed a trend of decreasing anti-*Pf*CSP IgG responses with increasing age, but found no evidence of protection in any of the age strata 1–2, 3–6, or 7–12 years. In contrast to *Pf*CSP-based vaccines, the causal link between antibody response and protection in *Pf*SPZ vaccines is weak, as the immune response to these vaccines is more complex, more individual, and primarily includes cellular effectors.[165] Intuitively, a further increase in *Pf*SPZ dose might help to improve efficacy, a strategy that has been successful in the development of *Pf*SPZ vaccines; however, cumulative evidence shows that doses beyond 9×10^5 *Pf*SPZ instead decrease efficacy.[166,167] Unfortunately, the absence of cellular immune samples limits our ability to further examine the mechanisms underlying the observed lack of protection despite the induction of anti-*Pf*CSP IgG responses. Genetic sieve analysis is ongoing to assess local parasite diversity and its potential effects on vaccine efficacy.

This trial was, to our knowledge, the first to measure *Pf*SPZ Vaccine efficacy in children aged 1–12 years and the first to use *Pf*SPZ Vaccine in Gabon. Comparing our results with upcoming data from other regions and presumptively treated participants will be of interest.

In conclusion, *Pf*SPZ Vaccine in a three-dose regimen of 9×10^5 *Pf*SPZ, administered by direct venous inoculation at days 1, 8, and 29 without preimmunisation pretreatment, was safe and well tolerated in children in Gabon aged 1–12 years, and did not show efficacy against naturally acquired *P*

161 – Idem 113.

162, 163 – Idem 116.

164 – Idem 115.

165 – Idem 110.

166 – Idem 125.

167 – Jongo SA, Church LWP, Mtoro AT, et al. Increase of dose associated with decrease in protection against controlled human malaria infection by PfSPZ Vaccine in Tanzanian adults. Clin Infect Dis 2020; 71: 2849–57.

falciparum infection. A clinical trial to assess the effect of presumptive treatment on the same vaccine regimen and in a similar age group is ongoing. This study will provide crucial data for all *Pf* SPZ vaccines, including the late liver stage-arresting genetically attenuated *Pf* SPZ-LARC2 Vaccine, which is being assessed in Burkina Faso. Nevertheless, we conclude that high-level efficacy can be achieved only with *Pf* SPZ vaccines that provide robust immunity against the late liver stage.

Contributors

BM, MBBM, STA, TLR, SLH, and PGK designed the trial. BKLS, TLR, LWPC, SLH, and NKC provided the investigational products and were responsible for immunological analysis. JBo led the main data analysis. AA managed the data and contributed to the interpretation of the results. AA, ALK, AM, JBi, and EM performed the clinical work and provided input on data analysis. STA, BM, AA, MBBM, AL, and ME designed and conducted exploratory investigations. STA was the principal investigator. PGK, AK, BM, LWPC, TLR, and SLH supervised the trial. All authors contributed to the analysis and writing of the manuscript. STA, JBi, JBo, LWPC, and BM directly accessed and verified the underlying data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

NKC, BKLS, TLR, LWPC, and SLH are employees of Sanaria, the owner and sponsor of the Pf SPZ Vaccine. STA (through CERMEL), TLR and SLH (through Sanaria), and BM (through the University of Tübingen) received grants through their respective institutions to conduct this trial. All other authors declare no competing interests.

Data sharing

De-identified individual-participant data and a data dictionary defining the dataset will be made available after publication of the primary outcomes of this trial. The study protocol, statistical analysis plan, and informed consent form will also be available. Data and supportive documents will be made available on request to admin@cermel.org. Data will be shared, preferably for secondary analyses, with the support of the principal investigator after a proposal summarising its use is approved. A data sharing agreement must be signed before data are shared.

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