Randomised trial of efficacy of SPf66 vaccine against Plasmodium falciparum malaria in children in southern Tanzania

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Summary
Effective, safe antimalarial vaccines have proved elusive. The synthetic polypeptide SPf66 vaccine is based on pre-erythrocytic and asexual blood-stage proteins of Plasmodium falciparum. We report here a randomised double-blind placebo-controlled trial of the efficacy of the SPf66 vaccine against clinical P falciparum malaria in Ilde, southern Tanzania, an area of intense perennial malaria transmission.

586 children aged 1–5 years received three doses of vaccine (n=274) or placebo (n=312). The incidence and density of parasitaemia were assessed through repeated cross-sectional surveys on subgroups of children. Morbidity was monitored over a 1 year period through passive case detection in all children plus active case detection in a subgroup of 191. An episode of clinical malaria was defined as measured fever (≥37.5°C) and parasite density >20 000/μL.

No severe side-effects were seen and the frequency of mild side-effects after the third dose was less than 6%. The vaccine was highly immunogenic and after three doses all vaccine recipients had detectable anti-SPf66 antibodies: the geometric mean index of response was 8·3 in the vaccine group and 0·7 in the placebo group. The incidence of parasitaemia was similar in both groups. 123 children had at least one episode of clinical malaria during the follow-up period after the third dose and annual incidence rates were 0·25 in the vaccine group and 0·35 in the placebo group. Estimated vaccine efficacy was 31% (95% confidence interval 0–52%; p=0·046). After the third dose there were 6 deaths among the study cohort (1 vaccine, 5 placebo). This study confirms that SPf66 is safe, immunogenic and reduces the risk of clinical malaria among children exposed to intense P falciparum transmission.

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See Commentary page 1172

Introduction
Malaria, especially that caused by Plasmodium falciparum, is the most important parasitic disease of man. It causes more than 400 million clinical cases and between 1 million and 3 million deaths per year, mainly among young children and pregnant women in sub-Saharan Africa. Malaria is endemic in 103 countries, in which over half the world’s population live.

The search for a vaccine against malaria has been long, partly due to the complex life cycle of the parasite, an incomplete understanding of the mechanisms of effective immunity, and a lack of surrogate measures of protection and of animal models. Various molecules from the pre-erythrocytic, asexual blood, and sexual stages have been characterised, and some may be promising vaccine candidates. However, the processes of antigen selection and vaccine development are complex and the best methods for selecting vaccines for trials remain unknown. Although the first attempt to immunise man was in 1936 it was not until the 1970s that findings in rodent and simian models were successfully applied to human malaria, and volunteers immunised with irradiated sporozoites were shown to be protected against P falciparum and P vivax infection. However, recombinant and synthetic vaccine candidates derived from pre-erythrocytic stage antigens, in particular the repeat sequence of the circumsporozoite protein, have not been protective in human trials.

Vaccines derived from asexual-blood-stage antigens are of special interest in Africa, because they may mimic the development of natural immunity in children living in endemic areas. Furthermore, such vaccines may induce long-term immunity because of natural boosting. The chimaeric protein SPf66 was the first such vaccine to be tested in man. SPf66 is a synthetic hybrid polymer solubilised in sterile saline solution and adsorbed onto aluminium hydroxide. The monomer unit is a chemically synthesised peptide of 45 aminoacids which contains aminoacid sequences derived from three asexual-blood-stage proteins (83, 55, and 35 kDa) linked by Pro-Asn-Ala-Asn-Pro (PNANP) repeat sequence of the circumsporozoite protein, have not been protective in human trials.4 5

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The Kilombero SPf66 trial in southern Tanzania is the first trial of the vaccine outside Latin America, and the first in an area of intense perennial transmission. Preliminary studies to evaluate the safety and immunogenicity of the vaccine among small groups of non-immune and semi-immune adults and children began in July, 1992 (groups Ia, Ib, and II). No adverse effects were found and the vaccine was well tolerated and
immunogenic. A phase III trial was then launched to determine the efficacy of the vaccine in preventing clinical malaria in African children living in this area. The rationale and design of these trials are described elsewhere. This paper is the first report of efficacy results from the phase III trial.

**Population and methods**

**Study area and population**

The study was done in the village of Idete (08° 5' S; 36° 30' E), about 20 km west of Ifakara, southern Tanzania. The village lies south of the Udzungwa Mountains, at the northern edge of the alluvial plain of the Kilombero River at 270 m. The main rains start in March and extend through to May, and the short rains occur in December and January. A cool dry season follows the long rains, in June and July. Annual rainfall (August, 1993 to July, 1994) was 1417 mm.

The Kilombero Valley is an area of intense and perennial malaria transmission. *Anopheles gambiae* sp and *A funestus* are the two main vectors. Although mosquito densities and exposure are seasonal, the prevalence of symptomatic *P falciparum* parasitaemia is high and shows no marked seasonality. 80% of infants are infected by age 6 months and data from an adjacent village indicate that, on average, everyone receives more than 300 infective bites per year. Transmission of the three other species of human malarials is low and unstable. Malaria control in the to ensure positive identification even if the child's card was forgotten or lost.

**Study design**

The primary objective of this randomised double-blind, placebo controlled trial was to determine whether three doses of SPf66 reduce the incidence of clinical episodes attributable to malaria or the prevalence and intensity of parasitaemia in children aged 1-5 years, and to estimate the protection achieved. The target sample size of 600 children was chosen to give the study at least 90% power to detect a 50% reduction in the incidence of clinical malaria during the year after administration of the third dose, at a 5% significance level. An analytical plan written by the investigators was agreed with four independent trial monitors before the code was broken.

Children were randomised individually to receive vaccine or placebo. The code, drawn up and held by the monitors, was released to the investigators on Aug 8, 1994, when a copy of the study data had reached the monitors. Children were further randomised by household of residence to one of three follow-up groups (figure 1), with target sample sizes of 200 per group. Parasitological surveillance was carried out through repeated cross-sectional surveys in groups III and IV to estimate the efficacy of the vaccine in reducing the incidence, prevalence, and intensity of parasitaemia. Active case detection was done among children in group V and children from all three groups were monitored for morbidity through passive case detection.
Screening, informed consent, and immunisation

Institutional and national ethical clearances were obtained from the Medical Research Coordinating Committee of the Tanzanian National Institute for Medical Research and from all participating institutions and sponsors. The trial started in February, 1993, following the monitors’ confirmation that the vaccine had been safe and immunogenic in preliminary studies.14 Village meetings were held to explain the purpose of the trial. Following initial consent, children were called in small groups to key posts throughout the village for screening. Mothers or guardians were given a copy of the consent form in Kiswahili, which included information on procedures and potential risks involved with the trial, including the use of a placebo control. This was read out by a trained senior field assistant who answered any questions the mothers had. The children’s identity and date of birth were checked, a brief medical history was taken, and a physical examination was done. Children were considered eligible if they had no history of allergies leading to medical consultation and treatment, and no acute condition warranting hospital admission or any chronic condition which might make them unsuitable. A fingerprick blood sample was taken into a heparinised microtainer (Becton Dickinson), from which a microcapillary tube was filled, and the packed cell volume (PCV) was measured. If the PCV was less than 25% the child was not enrolled. Children were given a single dose of sulfadoxine-pyrimethamine (25 mg sulfadoxine and 0.75 mg pyrimethamine per kg body weight) before every immunisation in order to clear blood stage P falciparum infections. Children who satisfied all inclusion criteria were asked to attend for immunisation 2 weeks later. On that day, the informed consent form was again read to mothers or guardians. Their understanding of the implications of trial participation was checked by their answers to three questions before the form was signed by the reader and a village elder, who acted as a witness.

Children were reviewed by a physician to check for exclusion criteria. Syringes were drawn from a cold box and the number of the vial was recorded and checked by a witness. SPf66 and placebo were injected subcutaneously with a 25G needle in the left upper deltoid area for the first and third dose, and right deltoid for the second. Vaccine doses were planned for weeks 0, 4, and 26 (figure 1). After each dose, children remained under medical surveillance for one hour with immediate access to portable resuscitation equipment, and were regularly monitored for side-effects. Children then returned home and parents were asked to report subsequent side-effects to the trial medical team on call at Idete dispensary.

Clinical and parasitological follow-up

Clinical and parasitological follow-up comprised both active and passive case detection and cross-sectional malarialogical surveys. Passive case detection, based at Idete dispensary, was started in February, 1993. Idete dispensary was refurbished by the project, and supplies of essential drugs were provided throughout the study. All children attending the dispensary because of a perceived illness were screened by project medical personnel, who provided round-the-clock cover. Children were identified, and axillary temperatures checked with an electronic thermometer (MBO, Munich, Germany). If the temperature was 37.5°C or greater or if fever during the previous 24 h was reported, a fingerprick blood sample was collected into a heparinised serum separator microtainer for PCV measurement, and thick and thin blood films were prepared. The children were then diagnosed and treated by the routine medical services at the dispensary. Quality control by senior project personnel included random checks on children leaving the dispensary, to confirm the accuracy of the system. Hospital admissions of study children at the St Francis Designated District Hospital, Ifakara, were monitored daily to detect severe illness and side-effects.

Parasitological surveillance was carried out through cross-sectional surveys in groups III and IV, to estimate the efficacy of the vaccine in reducing the incidence, prevalence, and intensity of parasitaemia. Children in group III had blood samples taken through fingerprick at defined time-points between doses 2 and 3 and samples from children in group IV were taken at similar times after the third dose (figure 1). At these surveys, any sick children were referred to the dispensary for diagnosis and treatment. All children in group V were followed by active case detection (figure 1). Children were visited at home weekly by project field assistants, from 4 weeks after the second dose. A brief morbidity questionnaire was administered and the axillary temperature recorded with an electronic thermometer. If fever in the previous 24 h was reported or if the temperature was 37.5°C or higher, thick and thin blood films were prepared, and the mother was advised to take the child to the dispensary. Quality control included weekly repeat visits by senior project personnel to a 10% random sample of children and weekly reallocation of field assistants to different village areas. Thermometers were checked in a water bath against a standard mercury thermometer every fortnight.

As a measure of drug pressure the level of chloroquine consumption was monitored throughout the study period. Urine samples were collected from group V children at week –2 and week 48, and urine from 50 children was collected every month through a randomised procedure that ensured that at least one sample from each child was collected over a one year period.

Cross-sectional surveys and active case detection ensured close demographic surveillance during the study period. Children who did not have any contact with the study team for more than one month were visited by a project field assistant to check residence.

Immunogenicity and laboratory methods

Blood samples were taken from group V children on the day of the first dose (week 0) and 4 weeks after the third dose to determine the immune response. Blood was collected by fingerprick into heparinised microtainers, centrifuged at 3000 rpm for 3 min, separated, and stored without preservatives at −70°C.

No serological assays were done until the code was broken. IgG levels against the whole polymeric SPf66 peptide were measured by the Falcon Assay Screening Test ELISA (Becton Dickinson Labware, Oxnard, CA, USA).19 Immunofluorescent antibody titres were also measured.13

Thick and thin blood films were air dried, stained with Giemsa, and read on a light microscope (Wild-Heerbrug, Switzerland) with a ×50 oil immersion lens and ×10 eyepieces. Parasite density was assessed by counting the number of asexual stage parasites per 200 leucocytes. Slides were declared negative only after 200 leucocytes had been read. Parasite numbers were converted to a count/μL by assuming a standard leucocyte count of 8000/μL.4 All slides were read twice independently, and a
third time if the ratio of densities from the first two was greater than 1.5 or smaller than 0.67 or if there was a discrepancy in positivity. If less than 30 parasites were counted a third reading was done if the difference in the number of parasites was greater than 10. The definitive result was based on the majority verdict for positivity and the geometric mean of the positive densities for positive slides.

PCV was measured in heparinised microcapillary tubes using a microhaematocrit centrifuge. Urine samples were tested for chloroquine by thin-layer chromatography.11 Haemoglobin was determined using a microhaematocrit centrifuge. Urine samples were tested for positivity and the geometric mean of the positive densities for positive slides.

Vaccine efficacy based on comparison of incidence rates of clinical episodes between vaccine and placebo was calculated using the standard formula 1—(I/Iv), where Iv and Ip are the incidence rates in vaccine and placebo groups, respectively. The primary analysis considered passive case detection follow-up starting four weeks after dose 3. The imbalance in the distance between home and dispensary is also small but this too has a confounding effect on estimates of efficacy from passive case detection. The imbalance between the groups in age is small but could confound estimates of efficacy because the risk of clinical malaria diminishes rapidly with age. The imbalance in the distance between home and dispensary is also small but this too has a confounding effect on estimates of efficacy from passive case detection.

The main analysis of efficacy is restricted to the 586 children (274 vaccine, 312 placebo) who were aged 1-5 years on March 1, 1993 and who received all three doses. Thus 43 children received the first but not the third dose: 4 refused to come to be screened, 5 children were too sick to attend, and 3 parents refused. Of the 664 children screened, 8 were excluded (1 was on tuberculosis treatment, 1 had a history of asthma, and 6 had a PCV less than 25). On the day of first vaccination, a further 25 children were excluded (16 were temporarily away, 1 had migrated, 4 refused to take part, and 1 had died, and 3 were excluded on medical grounds [1 pneumonia, 1 epilepsy, and 1 mixed mitral valve disease]). 631 children received the first dose, and 624 (99%) and 588 (93%), respectively, of those received the second and third doses. Thus 43 children received the first but not the third dose: 4 refused (1 vaccine/3 placebo), 2 were withdrawn due to illness (0/2), 6 died (2/4), and 31 moved away (17/14). 1 child received doses 1 and 3 but not dose 2, and 1 child was found subsequent to vaccination to have been aged wrongly and has been excluded from the analysis.

**Study cohort**

The main analysis of efficacy is restricted to the 586 children (274 vaccine, 312 placebo) who were aged 1-5 years on March 1, 1993 and who received all three doses. These children form the study cohort (table 1), and the two groups are well balanced except for age and distance between home and dispensary. The imbalance between the groups in age is small but could confound estimates of efficacy because the risk of clinical malaria diminishes rapidly with age. The imbalance in the distance between home and dispensary is also small but this too has a confounding effect on estimates of efficacy from passive case detection.

**Results**

**Screening and immunisation**

The census identified 789 children who would be aged between 1 and 5 years on March 1, 1993. The 672 children living nearest the centre of the village were asked to come to be screened. 5 children were too sick to attend and 3 parents refused. Of the 664 children screened, 8 were excluded (1 was on tuberculosis treatment, 1 had a history of asthma, and 6 had a PCV less than 25). On the day of first vaccination, a further 25 children were excluded (16 were temporarily away, 1 had migrated, 4 refused to take part, and 1 had died, and 3 were excluded on medical grounds [1 pneumonia, 1 epilepsy, and 1 mixed mitral valve disease]). 631 children received the first dose, and 624 (99%) and 588 (93%), respectively, of those received the second and third doses. Thus 43 children received the first but not the third dose: 4 refused (1 vaccine/3 placebo), 2 were withdrawn due to illness (0/2), 6 died (2/4), and 31 moved away (17/14). 1 child received doses 1 and 3 but not dose 2, and 1 child was found subsequent to vaccination to have been aged wrongly and has been excluded from the analysis.

**Table 2: Cumulative incidence and density (parasites/μL) of parasitemia among incident cases after second and third dose of vaccination**

<table>
<thead>
<tr>
<th>Weeks from first dose</th>
<th>Vaccine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No positive</td>
<td>Density†</td>
</tr>
<tr>
<td>After dose 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20/42 (48%)</td>
<td>1960 (599-8446)</td>
</tr>
<tr>
<td>12</td>
<td>29/42 (69%)</td>
<td>3746 (894-11 546)</td>
</tr>
<tr>
<td>16</td>
<td>30/42 (93%)</td>
<td>1984 (424-10 213)</td>
</tr>
<tr>
<td>24</td>
<td>42/42 (100%)</td>
<td>2892 (934-12 696)</td>
</tr>
<tr>
<td>After dose 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>11/65 (17%)</td>
<td>1657 (131-5675)</td>
</tr>
<tr>
<td>34</td>
<td>24/65 (37%)</td>
<td>745 (272-5722)</td>
</tr>
<tr>
<td>38</td>
<td>47/65 (72%)</td>
<td>952 (365-5815)</td>
</tr>
<tr>
<td>50</td>
<td>55/65 (85%)</td>
<td>2299 (606-5788)</td>
</tr>
<tr>
<td>73</td>
<td>63/65 (97%)</td>
<td>1716 (642-6676)</td>
</tr>
</tbody>
</table>

Numbers positive represent cumulative incidence in those who were negative 4 weeks after each dose and who had complete data.

Median of positive slides among those who had been negative 4 weeks after each dose, inter (first to third) quartile range shown in parentheses.
An episode is defined by fever 37.5°C and parasites >20 000/μL.

Table 3: Number of clinical episodes of *P. falciparum* per child as detected by passive case detection

<table>
<thead>
<tr>
<th>Episodes* per child</th>
<th>Vaccine (n=274)</th>
<th>Placebo (n=312)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between 2nd and 3rd dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>222 (81.0%)</td>
<td>238 (76.3%)</td>
</tr>
<tr>
<td>1</td>
<td>46 (16.8%)</td>
<td>62 (19.9%)</td>
</tr>
<tr>
<td>2</td>
<td>6 (2.2%)</td>
<td>10 (3.2%)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total episodes</td>
<td>283</td>
<td>252</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After 3rd dose</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>225 (82.1%)</td>
<td>238 (76.3%)</td>
</tr>
<tr>
<td>1</td>
<td>33 (12.0%)</td>
<td>52 (16.7%)</td>
</tr>
<tr>
<td>2</td>
<td>9 (3.3%)</td>
<td>18 (5.8%)</td>
</tr>
<tr>
<td>3</td>
<td>6 (2.2%)</td>
<td>2</td>
</tr>
<tr>
<td>Total episodes</td>
<td>273</td>
<td>210</td>
</tr>
</tbody>
</table>

*An episode is defined by fever 37.5°C and parasites >20 000/μL.

Table 3: Number of clinical episodes of *P. falciparum* per child as detected by passive case detection

Case detection because the risk of being diagnosed with malaria diminishes with distance from the dispensary. Both these confounding factors were taken into account when calculating adjusted estimates of vaccine efficacy.

After the third dose there were 20 withdrawals: 19 children moved away (10 vaccine, 9 placebo) and 1 refused to continue to participate (placebo).

Adverse effects

No severe adverse effects of vaccination were recorded and no children required medical care for the mild effects seen. After the first dose there were 25 children with mild or moderate induration (12 vaccine/13 placebo); after the second dose two contralateral inductions were noted (0/2); and after the third dose 5 children had erythema (3/2), 17 had induration (12/5), and 1 (1/0) had contralateral induration.

The number of immunised children attending the dispensary during the 2 days following each dose was similar (eg, for dose 3 3.3-6% in the vaccine group vs 3.2% in the placebo group; p=0.77).

Immunogenicity

The purpose of the limited serological data presented is simply to confirm vaccine immunogenicity. The relation between measured immune response and clinical protection has not yet been assessed. The prevalence of detectable anti-*P. falciparum* antibodies (IFAT) at baseline was similar in vaccine and placebo groups (49/81 [60%] vs 67/102 [66%]; p=0.47). The baseline prevalence of anti-SPf66 IgG antibodies was 74/81 (91%) among vaccinees and 90/102 (88%) among those receiving placebo (p=0.49). Geometric mean titres were also similar (table 1).

4 weeks after dose 3, all vaccine recipients assessed (74/74) and 88% (80/91) of placebo recipients had detectable SPf66 antibody (geometric mean titres 2782 in the vaccine group and 161 in the placebo). The geometric mean index of response was 8.3 in the vaccine group and 0.7 in the placebo group (p<0.001). IFA titres in the vaccine group also increased substantially after three doses of vaccine (geometric mean index of response 1.8 vs 0.7 [p<0.001]; geometric mean titres 207 and 17).

Mortality and hospital admissions

6 cohort children (1 vaccine/5 placebo) died during the 48 weeks of follow-up after the third dose. A further 6 children who had received first dose (2/4) died before the third dose was due at week 26. Thus 12 children died during the study: 5 deaths were in hospital. Hospital records and interviews with relatives suggest that 6 of the deaths were due to malaria (1 vaccine vs 5 placebo). 32 cohort children were admitted to the paediatric wards of St Francis Hospital during the full 17 months of follow-up (13 vaccine/19 placebo). Malaria was diagnosed in 10 (4/6).

Cross-sectional surveys

Table 2 shows the cumulative incidence of infection and median parasite density for incident infections separately for the periods after the second (group III) and third doses (group IV). Incidence rates were similar among vaccine and placebo recipients after both the second and third doses (age-adjusted hazard ratios 1.0 and 1.0). After the third dose, median parasite densities were lower among vaccinees than among placebo recipients. However, this difference was significant only at the last cross-sectional survey (p=0.03).

From cross-sectional surveys (figure 1) mean PCV was similar in vaccine and placebo recipients after the second and third doses (31.7% vaccine/31.8% placebo at week 24, 33.9%/33.5% at week 50, and 31.8%/31.9% at week 73). The proportion of children who had PCV of less than 25% was also similar in vaccine and placebo recipients after both second and third doses (not shown).

Chloroquine levels in urine were similar in vaccine and placebo recipients throughout the study. Chloroquine was found in 40/211 (19.0%) urine samples from SPf66 recipients and 47/239 (19.7%) of placebo recipients.

Passive and active case detection

The numbers of clinical episodes seen at the dispensary between second and third dose and after the third dose are shown in table 3. Vaccinees had fewer episodes than placebo recipients. Analyses of vaccine efficacy for the periods between second and third dose and after the third dose are shown in table 4. For the purpose of assessing efficacy, follow-up started 4 weeks after vaccination. The primary analysis, of first or only episodes of fever 37.5°C with parasitaemia over 20 000/μL after dose 3, gave a vaccine efficacy of 31% (95% CI 0–52%). After two doses of SP66, the vaccine efficacy was 22% (95% CI –12% to 46%). Kaplan Meier survival curves for the
Adjusted for age at episode and distance to dispensary.
≥37.5°C.
†Shown for comparison.

### Table 4: Annual incidence of first clinical episodes of *P. falciparum* and vaccine efficacy as established by passive case detection

<table>
<thead>
<tr>
<th>Period of follow-up</th>
<th>Case definition</th>
<th>Vaccine</th>
<th>Placebo</th>
<th>RR</th>
<th>Vaccine efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First (or only) episodes</td>
<td>Child-days at risk</td>
<td>Annual incidence rate</td>
<td>First (or only) episodes</td>
</tr>
<tr>
<td>Between 2nd and 3rd dose: Fever and &gt;20 000 parasites/µL</td>
<td>52</td>
<td>30 464</td>
<td>0-62</td>
<td>74</td>
<td>33 164</td>
</tr>
<tr>
<td>After dose 3: Fever and &gt;20 000 parasites/µL</td>
<td>49</td>
<td>72 052</td>
<td>0-25</td>
<td>74</td>
<td>78 046</td>
</tr>
<tr>
<td>Fever and &gt;0 parasites/µL</td>
<td>90</td>
<td>65 309</td>
<td>0-50</td>
<td>130</td>
<td>68 856</td>
</tr>
<tr>
<td>Fever and no parasites†</td>
<td>32</td>
<td>76 406</td>
<td>0-15</td>
<td>32</td>
<td>85 973</td>
</tr>
</tbody>
</table>

*Adjusted for age at episode and distance to dispensary.
†≥37.5°C.
‡Shown for comparison.

Discussion

This trial confirms that vaccination with the chimera protein SPf66 reduces the risk of malaria among children highly exposed to natural infection. The results complement those from Latin America, by providing evidence that the vaccine can induce partly effective immunity at the top end of the spectrum of malaria transmission intensity. The vaccine was safe in children highly exposed to *P. falciparum*, the incidence of mild or moderate local side-effects being low with no adverse effects requiring medical treatment. The vaccine was also highly immunogenic, leading to anti-SPf66 IgG titres similar to those observed in our preliminary studies, even though pre-existing antibodies against *P. falciparum* were common.

The vaccine incorporates the PNANP repeat sequence of the circumsporozoite protein. Despite the high immunogenicity of this vaccine the incidence of *P. falciparum* infections was similar in SPf66 and control groups, indicating that the vaccine does not induce effective anti-sporozoite immunity. In contrast, the asexual blood stage antigens in the vaccine appear to have induced or boosted immunity which reduced the incidence of clinical episodes of malaria. The increase in immunofluorescence antibodies after three doses suggests that anti-SPf66 antibodies raised after immunisation with SPf66 recognise native epitopes of *P. falciparum* merozoites.

The vaccine efficacy estimate was 31%, lower than the 50% level that the trial was designed to detect and resulting in wide confidence intervals. However, the primary case definition is less than 100% specific so vaccine efficacy is likely to be underestimated. Case definitions with lower parasitaemia cut-offs are even less specific and likely to result in greater bias. Too few episodes were recorded to establish clearly the duration or age dependence of protection induced by the vaccine. Indeed active case detection picked up far fewer episodes than we expected probably due to the improved health services in the village. Moreover, the trial was not intended (and did not have the power) to establish the impact of the vaccine on severe life-threatening malaria or death, though it is of interest that only 1 of the 6 deaths in the study cohort was in a vaccinee. We were not able to assess the severity of malaria episodes revealed by passive...
case detection. Our primary measure, the PCV, seems not to have been a good marker of malaria severity in this study since PCV was similar in symptomless children and those with clinical episodes.

SPf66 appears to be effective against P falciparum in both South America and Africa. The best estimates of efficacy in both areas are much higher than the prevalence of any one strain of parasites, a result which suggests that the mechanisms mediating protection must be effective against many strains. The vaccine may therefore mimic naturally acquired strain-transcending immunity.

In further analyses we will look at the effect of the vaccine on parasite densities and evaluate protection in relation to age and serological response. The partial protection reported in this paper in itself should encourage investigations to understand better the mechanisms involved, with the aim of improving efficacy. Our results suggest that two doses may give some protection, so further work on the vaccination schedule may be required. They also argue in favour of the approach to antigen selection followed by Patarroyo, and the use of synthetic chimaeric proteins as immunogens. In the absence of surrogate parasitological or immunological markers of protection, our results also highlight the importance of clinical and field trials in vaccine development.

The potential of SPf66 vaccine as a public health measure in Africa will be debated. Current malaria control strategies rely on partly effective tools such as chemotherapy for suspected cases and measures to reduce man-vector contact. The estimated efficacy of SPf66 is lower than that of most vaccines in use for other infections. However, since the burden of malaria morbidity and mortality is vast, measures with a moderate efficacy merit development.

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