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Evaluation of the SPf66 vaccine for malaria control when delivered through the EPI scheme in Tanzania

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Summary

BACKGROUND Malaria control programmes need to protect young children, who bear the brunt of malaria disease and death in Africa. The development of a vaccine is a priority if improved and sustained malaria control is to be achieved. The best use of a vaccine in Africa will be achieved if it can be delivered through the expanded programme of immunization (EPI). We conducted a trial designed to evaluate the efficacy of SPf66 vaccine for malaria control when delivered through the EPI scheme in Tanzania.

METHODS The study was a two-arm, double blind, individually randomized placebo controlled trial involving 1207 infants. The primary objective of the trial was to estimate the efficacy of three doses of SPf66 given at 1, 2 and 7 months of age in preventing clinical episodes of malaria. These were documented through a health facility-based passive case detection system.

RESULTS Among 1207 randomized children, overall compliance for third dose was 91%. SPf66 was safe, immunogenic and did not interfere with the humoral immune responses to EPI vaccines. There were 294 children among SPf66 recipients and 288 among placebo recipients with at least one malaria episode, yielding a vaccine efficacy estimate of 2% (95% CI: −16, 16; $P = 0.84$).

CONCLUSION This has been the first trial of a malaria vaccine among very young infants. It provides information on the safety of peptide vaccines administered at this early age as well as their capacity to induce immune responses without negatively interacting with EPI vaccines. Given the modest protection previously documented in older age groups and the lack of efficacy in younger infants, this vaccine in its current alum-based formulation does not appear to have a role in malaria control in sub-Saharan Africa. The lack of efficacy found in this trial also raises concerns about potential difficulties of inducing protective immune responses against malaria through immunization in infants.

keywords malaria vaccine, infants, EPI, SPf 66, *Plasmodium falciparum*

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Introduction

The current world malaria situation is probably no better than 30 years ago. *Plasmodium falciparum* malaria is still thought to be directly responsible for 1–3 million deaths of children every year in sub-Saharan Africa, as well as constituting a major obstacle to the economic development of the continent and a contributor to poverty. The last few years have witnessed a renewed awareness of the unacceptable state of malaria control worldwide and particularly in sub-Saharan Africa. This has led to the formulation of new initiatives, such as Roll Back Malaria, aiming to reduce the burden of

this disease with currently existing tools. However, the development of a vaccine is thought to be a priority if improved and sustained malaria control is to be achieved.

To date, only two antigens have been tested for efficacy among populations living in endemic areas and subject to natural exposure to *P. falciparum* (Graves 1997). One is SPf66, a multistage, multicomponent synthetic peptide. The Cochrane collaboration's systematic review currently includes six double blind, randomized placebo controlled trials (DB-RCTs) carried out in South America, Africa and Asia. Meta-analysis revealed overall vaccine efficacy estimates of 23% (95% CI: 12, 32) in reducing the incidence of the first or only attack of clinical *P. falciparum* malaria and of 39% (95% CI: 30, 47) for the total number of episodes. The review concludes that this vaccine, the first one to have reduced the risk of malaria among children living in endemic areas, deserves further investigation.

Malaria control programmes need to protect young children, who bear the brunt of malaria disease and death in Africa (World Bank 1993). The only functioning structure for the delivery of vaccines to children in most countries is the Expanded Programme of Immunization (EPI). The costs and logistics of setting up an alternative structure for the delivery of a new vaccine would be formidable.

As part of the programme to evaluate SPf66 as a potential tool for malaria control in Africa, initiated in 1992, we conducted a trial designed to evaluate the efficacy of SPf66 when delivered alongside the EPI. The rationale is based on the results of the previous trial in Tanzania (Alonso *et al.* 1994), which yielded a moderate but significant protective effect among 1–5 year-olds, and on the fact that none of the previous trials included children of an age compatible with delivery of the vaccine through the EPI.

Methods

Study site and population

The trial was conducted in the town of Ifakara, Kilombero district, southern Tanzania. The area is described in detail elsewhere (Menendez *et al.* 1997). The estimated number of infective bites per person per year in the Kilombero valley is more than 300 (Smith *et al.* 1993). The incidence of clinical malaria rises steeply after the first month of life (Kitua *et al.* 1996) and the incidence in infants attending local health facilities for malaria and severe anaemia, a common manifestation of malaria, are 0.7 and 0.6 episodes per child year, respectively (Menendez *et al.* 1997). Malaria control is based on prompt diagnosis and chloroquine treatment, although 60% of parasite strains show resistance at day 7 (Hatz *et al.* 1998). Government health facilities in Ifakara are limited to the Saint Francis Designated District Hospital (SFDDH) and the adjacent Ifakara Mother & Child Health (MCH) Clinic. SFDDH has 375 beds, 70 of which are for paediatric patients. In Kilombero District, 85% of all infants complete the full national EPI scheme (Font *et al.* unpublished observation) which consists of BCG/OPV at birth, DTP/OPV at age 1, 2 and 3 months, then measles at 9 months.

Study design

The study was a two-arm, double blind, individually randomized placebo controlled trial involving 1207 infants. An independent Data and Safety Monitoring Board (DSMB) regularly monitored all aspects of the trial following agreed Standard Operating Procedures.

Given that this was the first time the vaccine was given to very young infants, preliminary safety data were collected on the first 98 infants. The DSMB performed an analysis on these infants (groups 1 ($n = 46$) and 2 ($n = 52$)) before the remainder of the cohort (group 3 ($n = 1109$)) was recruited. Children in group 1 were excluded if they had low birth weight, any sign of neonatal infection or asphyxia, any gross congenital malformation or abnormal clinical parameters before vaccination. Children in groups 2 and 3 were only excluded if they required admission to hospital on the day of dose 1. No twins were recruited to avoid misidentification. A detailed description of the recruitment criteria of groups 1 and 2 and of the assessment of safety is presented elsewhere (Schellenberg *et al.* 1999).

The primary objective of the trial was to estimate the efficacy of three doses of SPf66 given at 1, 2 and 7 months of age in preventing clinical episodes of malaria. It was estimated that 1200 infants would provide 90% power to detect a vaccine efficacy of 25% at the 5% significance level (two-sided test). A clinical malaria episode in a child attending the health facility was defined as an axillary temperature ≥ 37.5 °C together with asexual *P. falciparum* parasitaemia of any density. This case definition has an estimated sensitivity and specificity of 100% and 88%, respectively (Menendez *et al.* 1997). The trial adopted a pragmatic approach by assessing the safety and efficacy of SPf66, as would be the case if it was in routine use and delivered through the EPI structure.

SPf66 is a synthetic hybrid polymer solubilized in sterile saline solution and adsorbed onto aluminium hydroxide (Moreno & Patarroyo 1989). The product used in this trial was the same as the one used in the previous Tanzanian study (Alonso *et al.* 1994). Both vaccine and placebo were bottled in identical 5-dose clear glass vials. Each dose of vaccine consisted of 500 mg of peptide adsorbed to 0.312 mg of aluminium hydroxide in a volume of 0.125 ml. The placebo consisted of aluminium hydroxide alone.

Informed consent, enrolment, blinding and vaccination

Information about the trial was given to mothers resident in Ifakara when they brought their children for their first contact with the EPI. Further information regarding the purpose of the trial, the procedures involved, potential risks and the use of placebo was given to parents/guardians immediately after the first dose of DTP/OPV was given. Comprehension was assessed with a set of standard questions. Following witnessed written informed consent, infants were recruited to the trial and received dose 1.

The DSMB coded vials with the letters A to F. A random list of the letters arranged in blocks of 6 was used to assign a child to receive one of these letters, 3 of which corresponded to SPf66. At the time of the first vaccination a child was assigned the next available unused letter. Although the SPf66 and placebo were very similar, a person not involved in any other aspect of the trial drew up doses from the corresponding vial and labelled syringes with the relevant letter in order to assure the blindness. These activities were performed with a screen between the vaccine handler and the vaccinator. The barrier prevented the vaccinator from seeing the preparation of the syringes and also prevented the vaccine handler from seeing the child to be vaccinated.

The vaccine was administered by subcutaneous injection into the opposite thigh to the site of the EPI vaccine given at the same time. SPf66/placebo doses one and two were given at the same time as the first two doses of DTP/OPV, one month apart. In order to preserve the immunization schedule used in previous trials, which administered the third dose 180 days after dose 1, a new vaccination contact was introduced for the third SPf66 dose, at the routine clinic visit for the 7-month weighing (Figure 1).

Follow-up

Identity photocards were issued after dose 1 and used at every subsequent contact. Children were examined twice in the hour after vaccination and symptoms and signs of local and systemic reactions were documented. Children were also examined after dose 3 of DTP/OPV and after measles vaccination. A morbidity questionnaire was completed for group 1 and 2 infants when visited at home, daily, for a week after vaccination. Parents were asked to attend the project clinic at SFDDH if the child experienced health problems at any time. The main purpose of this follow-up was to assess the incidence of adverse effects.

Since mid-1994 a hospital surveillance system has been operating at SFDDH and the MCH (Menendez *et al*. 1997). In brief, this system ensured that all study children who attended these health facilities were seen by project medical personnel who provided round-the clock clinical cover. At each consultation, a questionnaire was completed which documented signs and symptoms. If the temperature was ≥ 37.5 °C, or if there was a history of fever in the preceding

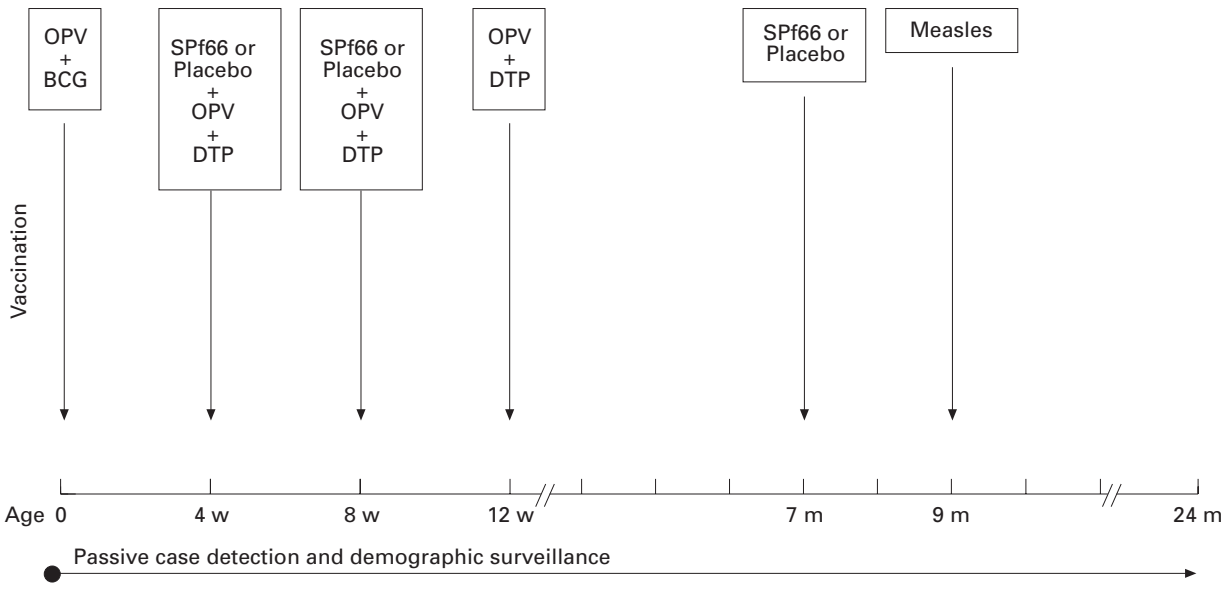

Figure 1 Study design and immunization schedule.

24 h, a finger-prick blood sample was used to prepare thick and thin blood films for malaria parasite examination and to measure the packed cell volume (PCV). For children who required admission a more detailed questionnaire was completed. Children with parasitologically confirmed clinical malaria were treated following national guidelines with oral chloroquine and/or sulphadoxine-pyrimethamine, or parenteral quinine if required.

Cross-sectional surveys were conducted to measure parasitaemia, packed cell volume (PCV), chloroquine consumption and haemoglobin trait at 8, 10 and 24 months of age. Serum samples were collected to assess serological responses to EPI vaccines and SPf66 as detailed elsewhere (Galindo *et al*. unpublished observation).

Hospital-based surveillance was complemented with a simple demographic surveillance system. Residence status and details of deaths occurring outside SFDDH were obtained through home visits either monthly (groups 1 and 2) or bimonthly (group 3). To estimate the distance from the child's home to the SFDDH, the position of each house was recorded using a GeoExplorer II Global Positioning System (Trimble, UK) with differential correction.

Laboratory methods

Thick and thin blood films were stained and read according to standard, quality-controlled procedures (Alonso *et al*. 1994) In short, blood slides were read twice by different readers who were blind to the other slide result. A third reading was done pending on whether there was a difference in *Plasmodium falciparum* positivity or the ratio of the densities was > 1.5 or < 0.67. If less than 30 parasites were counted and the difference in the number of parasites was > 10, a third reading was done. The definitive parasite reading was based on the majority verdict for positivity and the geometric mean of the positive densities for positive slides. The packed cell volume (PCV) was measured using a Hawksley haematocrit reader after centrifugation of the microcapillary tubes. Haemoglobin electrophoresis was performed on cellulose acetate strips on samples collected at 8 months of age. Urine was tested for 4-aminoquinolines using high-performance thin-layered chromatography (HPTLC-Alufolien Kieselgel 60 ^{R} Merck, Darmstadt, Germany). Antibodies against the SPf66 construct were determined using a FAST ELISA, as described by Teuscher *et al*. (1994), in a random selection of serum samples collected one month after the third dose. These assays were run blind after the code was broken. Humoral responses to some EPI vaccines were measured in the first 100 serum samples collected from infants aged 4 months (for polio, diphtheria and tetanus) and 10 months (for measles). Antibodies were determined following procedures described elsewhere (Galazka 1990).

Data management and statistical methods

Data were double-entered into FoxPro databases (Microsoft Corporation, US). Range, internal consistency and referential integrity checks were performed. All data collected up to 30 September 1998 was included. The files were cleaned, locked and handed to the DSMB in exchange for the code. A detailed analytical plan was drawn up by the investigators and approved by the DSMB before the code was handed over.

The primary efficacy analysis was based on Cox regression models of the risk of first or only episode of clinical malaria starting 30 days after dose 3 until the end of the study or censoring due to withdrawal or death. Vaccine efficacy (VE) was estimated from the hazard ratio (HR) as $VE = 100(1-HR)\%$. Unadjusted vaccine efficacy estimates were compared with efficacy estimates adjusted for each baseline characteristic in turn using the ratio of the two. Adjustments were made in the final model where adjusted and unadjusted estimates differed by 15% or more. The proportional hazards assumption was assessed using Cox regression models with time-dependent covariates.

Secondary analyses included a sensitivity analysis of case definitions based on different parasite density cut-off as well as an 'intention to treat' analysis of all children randomized, from dose 1 until the end of the study or death, using Poisson regression models. For multiple malaria episodes and all admissions to hospital, Poisson regression models with random effects were used to take account of between- and within-child variation (Clayton 1998). As the probability of an episode may depend on the previous history of events, the number of previous episodes was included in the model as a time-dependent covariate using the log (previous + 1) transformation (Lindsey 1995). For multiple malaria episodes, children were not considered at risk for 30 days after the start of each episode. The analyses were done using STATA statistical software (Stata Corp. 1997).

Results

Figure 2 shows the trial profile. Baseline characteristics of the study groups were similar (Table 1). Overall compliance of SPf66/placebo was 98.3% and 96.5% for second dose and 91.5% and 98.0% for third dose, respectively. Compliances within 28 days of the expected date of immunization for doses 2 and 3 were 95% and 80%, respectively. Seventy-two children received dose 3 at the same time as measles vaccine.

Safety data are described in detail elsewhere (Schellenberg *et al*. 1999). The frequencies of adverse reactions, abnormal laboratory parameters (data not shown) outpatient attendance, hospital admissions and deaths (Figure 2) were similar in SPf66 and placebo recipients. One child had a systemic allergic reaction after the third dose of SPf66 which resolved

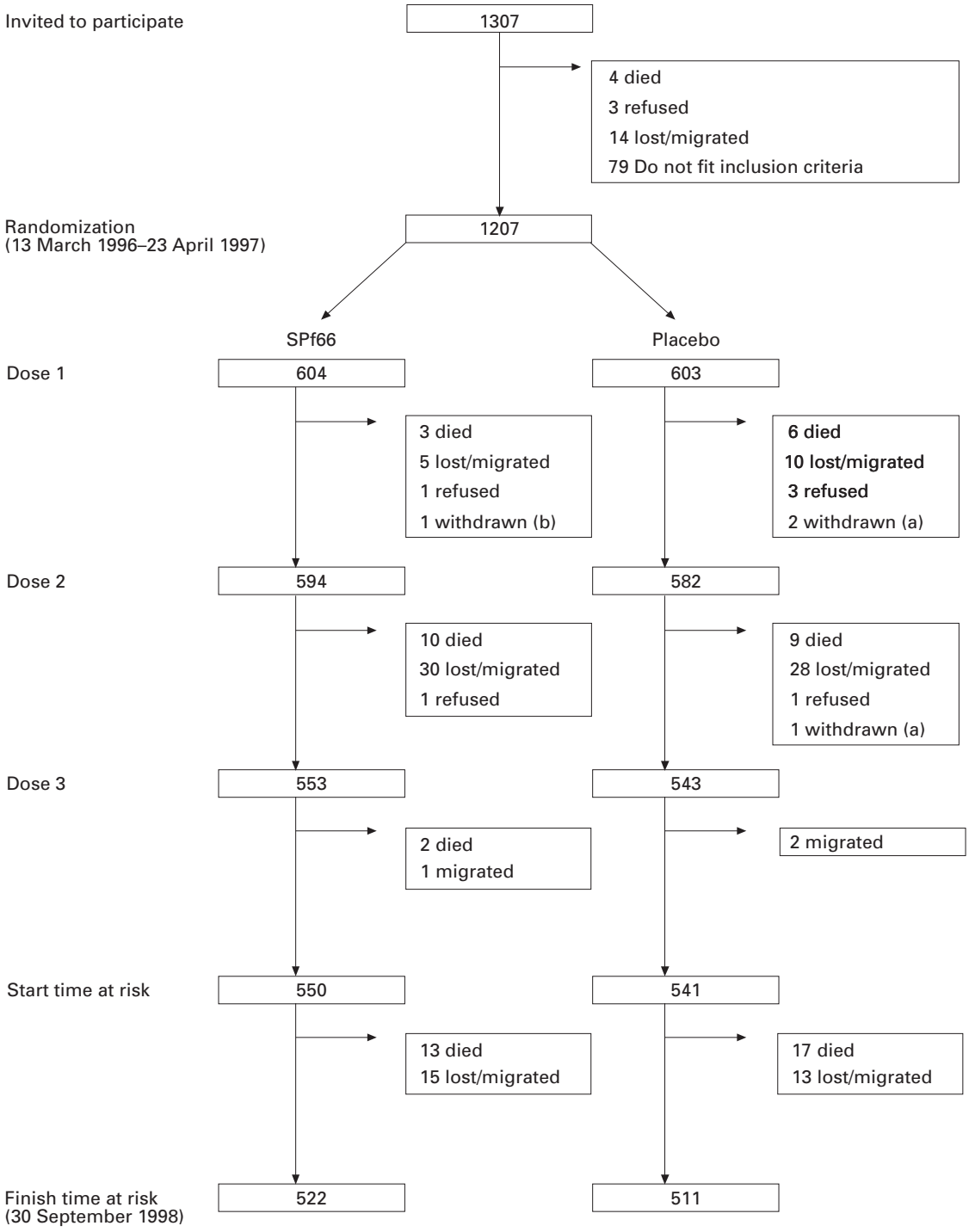

Figure 2 Trial profile. a, Clinical grounds on day of vaccincation; b, misvaccination.

Table 1 Baseline characteristics

	SPf66 (n = 550)*		Placebo (n = 541)*	
	n	%	n	%
Sex				
Male	252	(46)	259	(48)
Age (days) at randomization				
20 to 33	138	(25)	148	(27)
34 to 36	214	(39)	189	(35)
37 to 141	198	(36)	204	(38)
Weight/age z-score at randomization				
–5.8 to –0.41	182	(33)	166	(31)
–0.40 to 0.29	193	(35)	169	(31)
0.30 to 2.60	175	(32)	206	(38)
Distance to SFDDH (km) from child's home				
0.1 to 1.2	170	(31)	181	(33)
1.3 to 2.0	192	(35)	177	(33)
2.1 to 5.5	188	(34)	183	(34)
Age (days) at dose 3				
196 to 216	149	(27)	161	(30)
217 to 222	206	(37)	182	(34)
223 to 384	195	(35)	198	(37)
Breastfed at dose 3				
Exclusive	19	(3)	25	(9)
None	7	(1)	9	(2)
Mixed	524	(95)	507	(94)
Age (days) at measles vaccination				
214 to 278	144	(26)	129	(24)
279 to 282	213	(39)	184	(34)
283 to 484	181	(33)	201	(37)
Not vaccinated	12	(2)	27	(5)
Reported net use†				
Yes	233	(42)	217	(40)
No	198	(36)	206	(38)
Unknown	119	(22)	118	(22)
Chloroquine use‡				
Positive	15	(14)	16	(16)
Hb genotype¶				
AA/AF	82	(82)	87	(85)
AS/FS	18	(18)	15	(15)

*Denominator except for chloroquine use and Hb genotype; †At home visit after dose 3; ‡At age 10 months, n = 104 for SPf66 and 98 for placebo; ¶At age 10 months, n = 100 for SPf66 and 102 for placebo.

rapidly after a single dose of intramuscular promethazine. SPf66 was immunogenic with 98% (47/48) of SPf66 recipients positive for anti-SPf66 antibodies 1 month after dose 3 compared with 12% (6/50) of placebo recipients ($P < 0.001$). Geometric mean titres (GMT) in SPf66-seropositive children were also higher in SPf66 recipients (1181 in SPf66 *vs*. 64 in placebo; $P < 0.001$). All vaccine and placebo recipients developed protective titres for polio 3 (85/85). Few children tested did not develop protective titres against some other EPI vaccines; two infants (both SPf66 recipients) against diphtheria, 4 (3 placebo, 1 SPf66) against tetanus and 6 against measles (3 placebo, 3 SPf66). More detailed information on immunogenicity and interactions is reported elsewhere (Galindo *et al*. unpublished observation).

Table 2 Number of clinical episodes of *P. falciparum* per child from 30 days after dose 3

Episodes* per child	Vaccine (n = 550)	Placebo (n = 541)
0	256 (47%)	253 (46%)
1	147 (27%)	140 (26%)
2	85 (15%)	79 (15%)
3	35 (6%)	43 (8%)
>4	27 (5%)	26 (5%)
Total episodes	541	547

*Fever ≥ 37.5°C and parasites.

Vaccine efficacy

Between randomization and 30 days after dose 3, 29% (159/550) of the vaccine group and 32% (175/541) of the placebo group had one or more episodes of clinical malaria ($\chi^2 = 1.5$; $P = 0.22$). Table 2 shows the number and distribution of malaria episodes among vaccine and placebo recipients starting 30 days after dose 3. The primary analysis is restricted to 541 placebo and 550 SPf66 recipients who received all three doses and whose time at risk started 30 days after the third dose. There were 294 first or only episodes among SPf66 recipients and 288 among placebo recipients, yielding an adjusted vaccine efficacy estimate of 2% (95% CI: −16, 16; $P = 0.84$) (Table 3). The Kaplan Meier curve is presented in Figure 3. Secondary endpoints, including the intention-to-treat analysis as well as sensitivity analysis of different parasite density cut-offs, are presented in Table 3.

Discussion

We have reported the first trial of a synthetic peptide vaccine administered at 1, 2 and 7 months of age alongside the EPI vaccines. SPf66 was safe, immunogenic and did not modify the humoral responses to polio, tetanus, diphtheria and measles vaccines. However, SPf66 did not reduce the risk of clinical malaria among Tanzanian children living in this area of high perennial transmission.

This study was not intended to validate the results of any former trial but to progress down the critical path of vaccine evaluation for public health use. It was part of a comprehensive programme to evaluate the potential of SPf66 which had demonstrated a 31% efficacy among Tanzanian children aged

Table 3 Annual incidence of clinical episodes of *P. falciparum* malaria and estimated vaccine efficacy

		Vaccine			Placebo			RR	Vaccine efficacy (95% CI)				
Period of follow-up	Case-definition	Episodes	Child-years at risk	Annual incidence rate	Episodes	Child-years at risk	Annual incidence rate		Unadjusted	*P*	Adjusted	*P*	Regression mofrl
Primary analysis:													
From 30 days after dose 3	First/only episodes of fever* with parasitaemia	294	445.1	0.66	288	427.1	0.67	0.98	2.4% (−15,17)	0.772	1.7% (−16,16)	0.841	Cox†
Secondary analyses:													
From 30 days after dose 3	First/only episodes of fever* & parasitaemia > 5,000/μl	254	484.9	0.52	249	470.5	0.53	0.98	1.8% (−17,18)	0.836	1.8% (−17,18)	0.843	Cox†
	First/only episodes of fever*& parasitaemia > 20,000/μl	210	534.1	0.39	207	513.4	0.40	0.97	3.0% (−18,20)	0.754	3.2% (−17,20)	0.739	Cox†
	First/only episodes of severe anaemia (PCV < 25%)	106	621.7	0.17	93	619.5	0.15	1.12	−11.8% (−48,15)	0.431	−9.8% (−45,17)	0.510	Cox‡
	All admissions to SFDDH	725	713.9	1.02	767	697.1	1.10	0.91	9% (−3,20)	0.152	13.1% (−1,25)	0.062	Poisson¶
Intention to treat from randomization	All episodes of fever*with parasitaemia	763	1077.0	0.71	811	1061.7	0.76	0.92	7.7% (−5,19)	0.237	6.7% (−6,18)	0.275	Poisson¶
	All episodes of fever* & parasitaemia > 5,000/μl	602	1089.1	0.55	656	1074.0	0.61	0.89	10.6% (−4,23)	0.139	9.0% (−4,21)	0.174	Poisson¶
	All episodes of fever* & parasitaemia > 20,000/μl	420	1103.6	0.38	490	1086.7	0.45	0.83	16.5% (1,29)	0.035	13.8% (0,26)	0.058	Poisson¶

* ≥ 37.5 °C; † adjusted for weight-for-age at randomization, distance to SFDHH, and net use after dose 3; ‡ adjusted for weight-for-age at randomization; ¶ Random effects Poisson models, adjusted for the previous number of episodes as a time variable covariate.

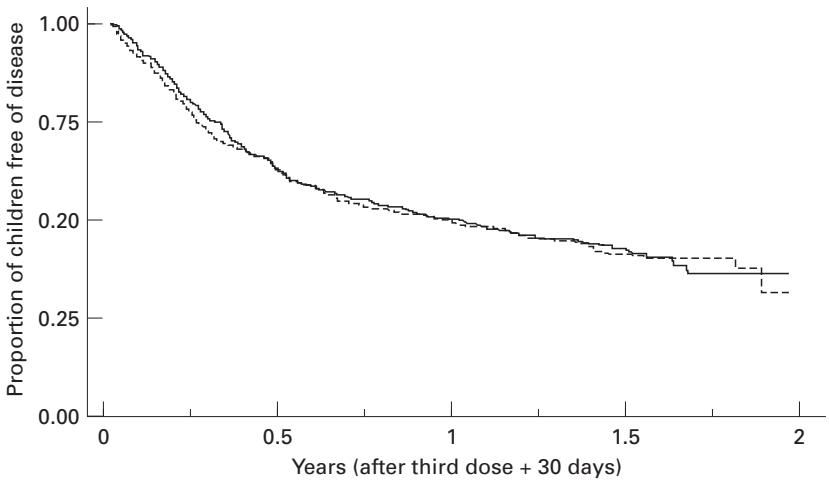

Figure 3 Kaplan Meier curve for first or only clinical malaria episodes. ——— SPf66; ----------Placebo.

1–5 years. The rationale of this trial was based on three considerations:

- the high risk of malaria morbidity and mortality during the first year of life, especially in areas of high transmission;
- the realization that a malaria vaccine will be of maximum use in Africa if it can be integrated in the current schedule of immunization;
- the level of protection documented in the first trial, although modest, still warranted further testing of this vaccine in the same setting.

Why has the vaccine failed to protect young infants when the same product in the same area given to older children reduced their risk of malaria? First of all, the confidence intervals for efficacy estimates from the two Tanzanian trials overlap. As a result it could be argued that the results are compatible and that the differences may be due to chance. However, in the first Tanzanian trial, all the results – including the primary outcome – consistently supported the finding of a positive result. These included efficacy against first or only malaria episodes, including different parasite cut-offs in the sensitivity analysis (Alonso *et al*. 1994) as well as multiple episodes persisting during extended follow-up (Alonso *et al*. 1996). There was also a 21% reduction in geometric mean parasite density in SPf66 recipients (Alonso *et al*. 1996) and a reduction in the number of concurrent *P. falciparum* infections (Beck *et al*. 1997). Just as the findings in the former trial were consistent and positive, the various indicators assessed in the current trial tended to corroborate the lack of efficacy in young infants. The role of chance cannot be excluded, but we consider it an unlikely explanation for the discrepant estimates of efficacy in the two Tanzanian trials.

Secondly, the two Tanzanian studies afford a unique opportunity to assess the importance of age in determining the efficacy of SPf66. The same vaccine was used in the same setting, but in younger children. The most likely explanation for the different results is that very young infants have different immunological reactions to SPf66, or possibly malaria antigens in general, rendering them less receptive to vaccination attempts. This age dependence in the response is a real possibility, as suggested by the lower titres reached among vaccinees in this trial or as is the case for pneumococcal polysaccharide vaccines (Temple *et al*. 1991). An analysis of the kinetics and the quality of antibody response is in progress. Should age, maturation of the immune system, interference with maternal antibodies or prior exposure be important determinants of the capacity to induce protective responses against malaria, these would have implications for malaria vaccine design and implementation strategies in the future.

SPf66 has been the most extensively tested malaria vaccine to date. Phase III trials have been carried out in South America, Asia and Africa. A full review of these results is beyond the scope of this paper, but are available from the Cochrane Collaboration (Graves 1997). In Africa, there has only been one other trial beside the two Tanzanian studies previously discussed. In The Gambia, an area of moderate and seasonal malaria transmission, a phase III trial was carried out among children aged 6–12 months at the time of the first dose. The lack of efficacy documented in that trial supports the notion that age may be an important determinant in inducing protective immunity with this vaccine.

In conclusion, we have conducted the first trial of a malaria vaccine among very young infants subject to intense *P. falciparum* malaria, and provided information on the safety of peptide vaccines administered to young infants as well as of their capacity to induce immune responses without negatively interacting with EPI vaccines. The documented lack of efficacy raises concerns about potential difficulties of inducing protective immune responses against malaria through immunization in infants. Given the modest protection documented

in older age groups and the lack of efficacy in the younger infants, this vaccine in its current alum-based formulation does not appear to have a role in malaria control in sub-Saharan Africa.

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