

# Long-Term Safety and Efficacy of the RTS,S/AS02A Malaria Vaccine in Mozambican Children

Jahit Sacarlal,<sup>1,2,5</sup> Pedro Aide,<sup>1,3</sup> John J. Aponte,<sup>1,5</sup> Montse Renom,<sup>1,5</sup> Amanda Leach,<sup>6</sup> Inácio Mandomando,<sup>1,3</sup> Marc Lievens,<sup>6</sup> Quique Bassat,<sup>1,5</sup> Sarah Lafuente,<sup>1,5</sup> Eusébio Macete,<sup>1,4</sup> Johan Vekemans,<sup>6</sup> Caterina Guinovart,<sup>1,5</sup> Betuel Sigauque,<sup>1,3</sup> Marla Sillman,<sup>7</sup> Jessica Milman,<sup>7</sup> Marie-Claude Dubois,<sup>6</sup> Marie-Ange Demoitie,<sup>6</sup> Joelle Thonnard,<sup>6</sup> Clara Menéndez,<sup>1,5</sup> W. Ripley Ballou,<sup>6,a</sup> Joe Cohen,<sup>6</sup> and Pedro L. Alonso<sup>1,5</sup>

<sup>1</sup>Centro de Investigação em Saúde de Manhiça (CISM) Manhiça, <sup>2</sup>Faculdade de Medicina, Universidade Eduardo Mondlane, <sup>3</sup>Instituto Nacional de Saúde and <sup>4</sup>Direcção Nacional de Saúde, Ministério de Saúde, Maputo, Mozambique; <sup>5</sup>Barcelona Center for International Health Research, Hospital Clinic/Institut d'Investigacions Biomèdiques August Pi i Sunyer, Universitat de Barcelona, Barcelona, Spain; <sup>6</sup>GlaxoSmithKline Biologicals, Rixensart, Belgium; <sup>7</sup>Program for Appropriate Technology in Health, Malaria Vaccine Initiative, Bethesda, Maryland

(See the editorial commentary by Bremen and Plowe, on pages 317–20, and the article by Kester et al, on pages 337–46.)

**Background.** We previously reported that the RTS,S/AS02A vaccine had an acceptable safety profile, was immunogenic, and demonstrated efficacy against *Plasmodium falciparum* malaria disease for 21 months.

**Methods.** We conducted a randomized, controlled, phase 2b trial of RTS,S/AS02A in 2022 Mozambican children aged 1–4 years. We now report safety results for all randomized subjects and vaccine efficacy (VE) findings for children in the Manhiça area over the 45-month surveillance period.

**Results.** During the surveillance period, the VE<sub>(2.5–45)</sub> (VE over months 2.5–45 of surveillance) against a first or only episode of clinical malaria disease was 30.5% (95% confidence interval [CI], 18.9%–40.4%;  $P < .001$ ), and the VE<sub>(2.5–45)</sub> against all episodes was 25.6% (95% CI, 11.9%–37.1%;  $P < .001$ ). When the same period was considered, the VE<sub>(2.5–45)</sub> for subjects protected against severe malaria was 38.3% (95% CI, 3.4%–61.3%;  $P = .045$ ). At study month 45, the prevalence of *P. falciparum* was 34% lower in the RTS,S/AS02A group than in the control group (66 [12.2%] of 541 patients vs 101 [18.5%] of 547 patients) ( $P = .004$ ).

**Conclusion.** These results show evidence that RTS,S/AS02A maintained protection during the 45-month surveillance period, and they highlight the feasibility of developing an effective vaccine against malaria. In combination with other malaria-control measures, such a vaccine could greatly contribute to reducing the intolerable global burden of this disease.

**Trial registration.** ClinicalTrials.gov identifier NCT00197041 and NCT00323622.

During the 20th century, economic and social development, together with antimalarial campaigns, have resulted in the eradication of malaria from large swathes of the planet, thereby reducing the percentage of the world's areas that are malaria prone from 50% to 27%. Nonetheless, given expected population growth, it is projected that, by 2010, one-half of the world's population—nearly 3.5 billion people—will be living in areas where malaria is transmitted [1]. Today, Africa continues to

absorb the brunt of the disease, with approximately 350–550 million clinical episodes and 700,00 to 1.6 million deaths occurring annually, mostly among children <5 years of age [1, 2].

The past decade has witnessed a renewed effort to study and control malaria. New tools are becoming available, and the development of a vaccine is considered to be a key component of future improved control activities.

Potential conflicts of interest: PATH Malaria Vaccine Initiative (MVI) supports the development and testing of a number of malaria vaccines that can be seen as competitors. A.L., M.L., J.V., J.T., M.-C.D., M.A.D., W.R.B., and J.C. are current or previous employees of GlaxoSmithKline Biologicals (GSK). A.L., W.R.B., M.-C.D., and J.C. own shares in GSK. Both J.C. and W.R.B. are listed as the “inventors” of patented malaria vaccines. However, neither individual holds a patent for a malaria vaccine. All other authors: no conflicts.

Financial support: Please see the Acknowledgments for information on financial support.

Engerix-B and Hiberix are trademarks of the GlaxoSmithKline group of companies. Prevenar is a trademark of Wyeth-Lederle.

<sup>a</sup> Employed by GlaxoSmithKline Biologicals at the time of the study.

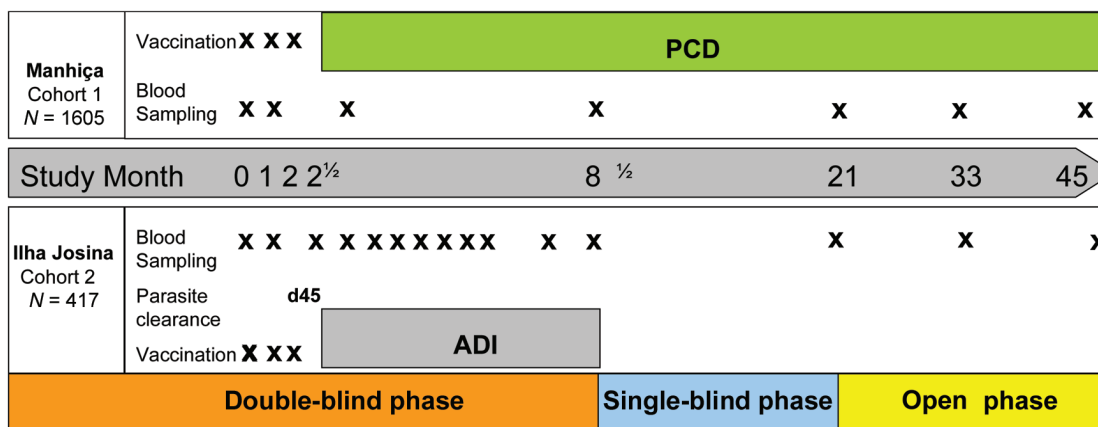
Received 14 October 2008; accepted 26 January 2009; electronically published 29 June 2009.

Reprints or correspondence: Dr. Jahit Sacarlal, Barcelona Center for International Health Research, Hospital Clinic, Universitat de Barcelona, C/Rosellón 132, 4<sup>o</sup> 08036 Barcelona, Spain (jahit.sacarlal@manhica.net).

The Journal of Infectious Diseases 2009;200:329–36

© 2009 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2009/20003-0003\$15.00

DOI: 10.1086/600119



**Figure 1.** Study design. ADI, active detection of information; d45, day 45; double-blind phase, study months 2.5–8; open phase, study months 21–45; PCD, passive case detection; single-blind phase, study months 8–21.

RTS,S (GlaxoSmithKline Biologicals [GSK]), a recombinant antigen, that is formulated with the AS02A Adjuvant System and that contains an oil-in-water emulsion and the immunostimulants QS21 (a triterpene glycoside purified from the bark of *Quillaja saponaria*) and 3D-MPL (3-deacylated monophosphoryl lipid A [MPL]), is currently the most clinically advanced malaria vaccine candidate in the world. RTS,S/AS02A specifically targets the preerythrocytic stage of *Plasmodium falciparum* and has been shown to confer protection against experimental *P. falciparum* infection, delivered via laboratory-reared infected mosquitoes, in immunized malaria-naïve volunteers and against natural infection in semi-immune adults [3–7].

Consecutive phase 1 trials in children aged 6–11 years and 1–5 years in The Gambia showed that the vaccine was safe, well tolerated, and immunogenic [3, 6, 8]. Short-term protection against infection (vaccine efficacy, 71% [95% confidence interval {CI}, 46%–85%] during the first 9 weeks of follow-up) was demonstrated in immunized adult men in The Gambia in 1998 [3]. Subsequently, a pediatric vaccine dose was selected and studied in a phase 1 trial of Mozambican children aged 1–4 years, in whom it was found to be safe, well tolerated, and immunogenic [9].

In 2004, we reported the first proof-of-concept study involving African children aged 1–4 years who were living in a *P. falciparum*-endemic area in Mozambique. During the first 6-months of follow-up in this double-blind, randomized, controlled trial, immunization with RTS,S/AS02A was associated with vaccine efficacy (VE) of 29.9% (95% CI, 11.0%–44.8%;  $P = .004$ ) against clinical malaria, 45% (95% CI, 31.4%–55.9%;  $P < .001$ ) against infection, and 57.7% (95% CI, 16.2%–80.6%;  $P = .019$ ) against severe malaria [10].

An extended follow-up showed that, at 21 months after the first dose, the risks of clinical malaria and severe malaria were reduced by 35.3% (95% CI, 21.6%–46.6%;  $P < .001$ ) and 48.6%

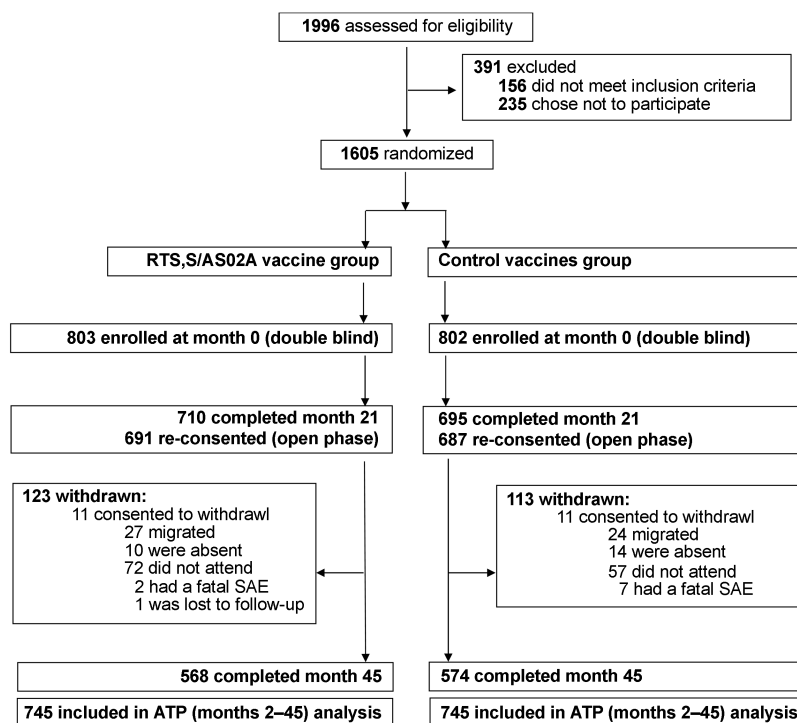
(95% CI, 12.3%–71.0%;  $P = .02$ ), respectively, in the RTS,S/AS02A group [11].

We recently completed a phase 1/2b clinical trial in infants living in a malaria-endemic area of Mozambique. Administration of RTS,S/AS02A, staggered with expanded program on immunization vaccines, showed that RTS,S/AS02A had a good safety profile was well tolerated and immunogenic, and was associated with a VE against new infection of 65.9% (95% CI, 42.6%–79.8%;  $P < .001$ ) [12].

Future deployment of any vaccine will depend on the level of VE and the duration of protection, both of which are critical elements of any target product profile. The present study reports the long-term safety and efficacy noted during 45 months of follow-up of Mozambican children who were 1–4 years of age at the time that they received a first dose of either RTS,S/AS02A or control vaccines.

## METHODS

**Study site.** The study was conducted at the Centro de Investigação em Saúde de Manhiça (CISM; Manhiça Health Research Centre) in Manhiça District, a rural area of Maputo Province, southern Mozambique, from April 2003 through May 2007. The characteristics of the area and the dates of malaria transmission have been described in detail elsewhere [13, 14]. Malaria transmission, mostly due to *P. falciparum*, is perennial, with marked seasonality. *Anopheles funestus* is the main vector, and the estimated entomologic inoculation rate for 2002 was 38 infective bites per person per year [10]. Combination therapy with amodiaquine and sulfadoxine pyrimethamine was the first-line treatment used for uncomplicated malaria during the first 2 years of the study, and it was replaced by the combination of sulfadoxine pyrimethamine plus artesunate in 2006. All antimalarial drugs were readily available at health care facilities



**Figure 2.** Trial profile for cohort 1. ATP, according-to-protocol analysis; SAE, serious adverse event.

in Mozambique throughout the study. Each participant received an insecticide-treated bednet during the study. Throughout the duration of the trial, indoor residual spray was promoted in the study area by the Mozambique Ministry of Health. Adjacent to the CISM is the Manhiça District Hospital, a 110-bed referral health care facility. The district health network consists of an additional 8 peripheral health care posts and another rural hospital.

**Study design.** This study is a phase 2b, randomized controlled trial to assess the efficacy, safety, and immunogenicity of 3 doses of the candidate RTS,S/AS02A malaria vaccine administered to children aged 1–4 years. The present study includes different follow-up periods (figure 1). The initial double-blind phase included study months 0–8.5. During this time period, and according to protocol, the investigators were unblinded, and a first analysis of safety and efficacy was performed and reported [10]. Study participants and case ascertainment mechanisms remained blinded, and follow-up was sustained, in accordance with protocol, in the single-blind phase occurring from study months 8.5 to 21 [11]. A subsequent new protocol was developed to expand follow-up of the safety and efficacy of the study cohorts from study months 21 to 45. The present study includes safety and efficacy data for the entire study period from month 0 to month 45.

A total of 2022 healthy children aged 1–4 years were enrolled to receive either the candidate malaria vaccine or a comparison control vaccine. The parents or guardians of all participants

provided written or thumb-printed informed consent before study enrollment. A member of the community acted as an impartial witness and countersigned the consent form to guarantee an adequate understanding of the study procedures by all guardians. Eligibility screening included a brief medical history, a physical examination, and blood sampling by finger prick for hematologic and biochemical tests. Children did not undergo screening tests for human immunodeficiency virus (HIV) infection. Hepatitis B surface antigen (HBsAg) status and anti-HBsAg antibody levels were assessed at baseline but were not criteria for exclusion from the trial. Other serologic markers of hepatitis B status were not assessed.

Children were randomized 1:1 to receive RTS,S/AS02A (in a 0.25-mL dose) or the control vaccines. The RTS,S/AS02A candidate vaccine was administered intramuscularly in the deltoid region of alternating arms, starting with the left arm, according to a 0-, 1-, and 2-month schedule. Children in the control group who were  $\geq 24$  months of age received 3 pediatric doses (0.5 mL) of hepatitis B vaccine (Engerix-B; GSK). Children  $< 24$  months of age received 2 pediatric doses of 7-valent pneumococcal conjugate vaccine (Prevenar; Wyeth Lederle Vaccines), which was administered at the first and third vaccinations, and 1 dose of *Haemophilus influenzae* type B vaccine (Hiberix; GSK Biologicals), which was administered at the second vaccination.

Children were enrolled in 2 cohorts to measure the VE against clinical malaria disease and malaria infection. In cohort

**Table 1. Percentage of Participants Reporting Serious Adverse Events (SAEs), as Classified by the *Medical Dictionary for Regulatory Activities (MedDRA)* [19] Primary System Organ Class and Preferred Term, over 45 Months of Follow-up (Intention-to-Treat [ITT] Analysis of Months 0–45)**

Finding	Control vaccine recipients with SAEs (n = 1010)		RTS,S/AS02A vaccine recipients with SAEs (n = 1012)	
	No. <sup>a</sup>	% <sup>b</sup> (95% CI)	No. <sup>a</sup>	% <sup>b</sup> (95% CI)
Subjects with ≥1 SAE <sup>c</sup>				
Reported	326	32.3 (29.4–35.3)	235	23.2 (20.7–25.9)
Reported and requiring hospitalization	199	19.7 (17.3–22.3)	159	15.7 (13.5–18.1)
SAEs reported and classified by <i>MedDRA</i> preferred term <sup>d</sup>				
Among all subjects	770	...	639	...
Among subjects requiring hospitalization <sup>d</sup>	525	...	454	...
Death				
Due to all causes	22	2.2 (1.4–3.3)	12	1.2 (0.6–1.2)
Excluding those due to trauma	18	1.8 (1.0–2.8)	11	1.1 (0.5–1.9)
Malaria related	5	0.5 (0.1–1.1)	1	0.1 (0.0–0.5)

**NOTE.** CI, confidence interval.

<sup>a</sup> No. of subjects who had ≥1 dose administered, were included in an ITT cohort, and reported the symptom at least once.

<sup>b</sup> Percentage of subjects who reported the symptom at least once.

<sup>c</sup> At least one symptom experienced (regardless of the *MedDRA* preferred term).

<sup>d</sup> Symptoms reported by a subject after administration of a given dose and classified by the same preferred term are counted once.

1 (from the Manhica area), 1605 participants were monitored using passive surveillance, to detect clinical episodes of malaria, and safety surveillance, until month 45. In cohort 2 (from the Ilha Josina village), 417 participants were monitored using active surveillance, to detect malaria infection through visits that started 14 days after administration of dose 3, continued every 2 weeks for 2.5 months, and then continued monthly for an additional 2 years. At the end of the single-blind phase, new informed consent was obtained to continue follow-up for 2 more years. Surveillance for this cohort was continued through a health care facility-based passive case-detection system, to monitor safety.

The protocol (Investigational New Drug no. BB-IND 10514) was approved by the Mozambican National Ethics Review Committee, the Hospital Clinic of Barcelona (University of Barcelona) Ethics Review Committee, and the PATH Human Subjects Protection Committee. The trial was conducted in accordance with International Conference on Harmonisation of Good Clinical Practice guidelines and was monitored by GSK. A local safety monitor and a data and safety monitoring board closely reviewed the conduct and safety data of the trial.

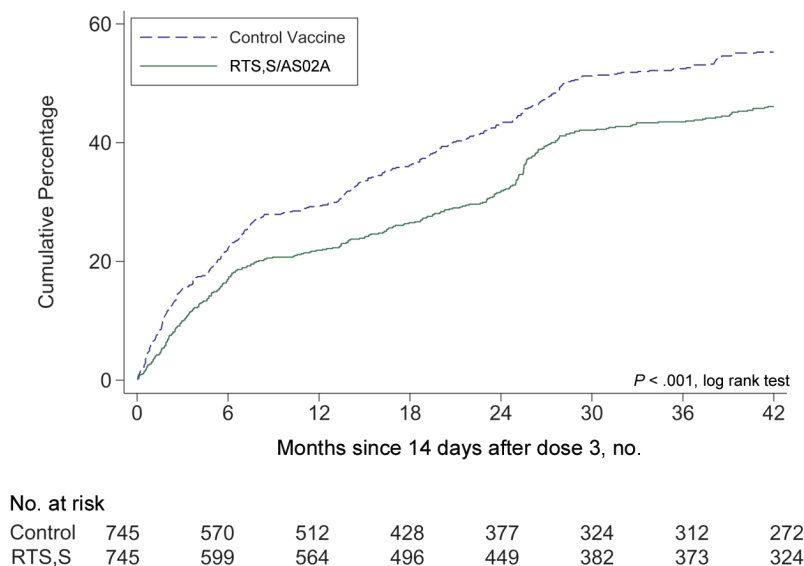
**Study procedures.** Vaccines were administered at the Manhica and Ilha Josina health care centers. Vaccine safety was evaluated using active and passive follow-up [15].

A serious adverse event (SAE) was defined as any medical event that resulted in death, was life-threatening, required inpatient hospitalization, or resulted in persistent or significant disability or incapacity. Investigators monitored participants

with SAEs until the event had resolved or until month 45 of surveillance. Deaths that occurred at home were investigated by a review of all available medical records and through a verbal autopsy.

**Statistical methods.** Safety analysis was based on intention-to-treat (ITT) analysis of study participants included in both cohorts 1 and 2 during months 0–45. Analyses of VE against clinical malaria were based on cohort 1 study participants who were compliant with study procedures (ie, the according-to-protocol [ATP] cohort for analysis) from month 2.5 to month 45 during the study period

A child with a clinical episode was defined as a child who presented to a health care facility with an axillary temperature of ≥37.5°C and *P. falciparum* asexual-stage parasitemia of ≥2500 parasites/μL (as per primary case definition). A child requiring admission to the hospital for malaria was defined as a child with *P. falciparum* asexual-stage parasitemia for whom malaria was judged to be the sole cause of illness or a substantial contributing factor. All cases of severe malaria were defined by the presence of asexual *P. falciparum* parasitemia in a severely ill child, with there being no other more-probable cause of illness. Severe malaria was defined by the presence of any the following conditions: severe malaria anemia (packed-cell volume, <15%), cerebral malaria (Blantyre coma score, <2), and/or severe disease of other body systems (eg, multiple seizures [≥2 generalized convulsions in the previous 24 h], prostration [defined as an inability to sit unaided], hypoglycemia [<2.2 mmol/L], clinically suspected acidosis, or circulatory collapse)



**Figure 3.** Kaplan-Meier curves for the cumulative proportion of children with  $\geq 1$  episode of clinical malaria.

[16]. All hospital admissions were independently reviewed by 2 groups of clinicians, to determine whether malaria was the cause of the admissions and whether the cause fulfilled the definition of severe malaria. Discrepancies were resolved by consensus.

For the efficacy analyses, except for analyses of hospital admissions, the time at risk was calculated with absences from the study area and antimalarial drug use both considered. For analysis of multiple episodes of clinical malaria, a subject was not considered to be susceptible for 28 days after the previous episode. After receiving malaria treatment, a child was not considered to be at risk for an arbitrary period of 28 days after receiving sulfadoxine pyrimethamine, 7 days after chloroquine alone, 7 days after quinine alone, 7 days after amodiaquine, and 20 days after artemether plus lumefantrine.

For the time to a first or only episode of clinical malaria, VE was assessed using Cox regression models and was defined as:  $(1 - \text{hazard ratio}) \times 100$ . The VE was adjusted for predefined covariates of age, bed net use, geographic area (administrative divisions), and distance from a health care center. Cox regression assumes proportional hazards throughout follow-up. This assumption was checked graphically by plotting per group the log of the cumulative hazard against the log of time [17], as well as by using a test based on the Schoenfeld residuals and time-dependent Cox regression models [18].

For multiple episodes of clinical malaria and hospital admission, the group effect was assessed using Poisson regression models with normal random intercepts, including the time at risk as an offset variable.

Differences in the proportions of children with at least 1 episode of severe malaria disease and the prevalence of asexual

*P. falciparum* at each cross-sectional survey were compared using Fisher's exact test. For severe malaria, VE was calculated as  $1 - \text{risk ratio}$ , with the exact 95% confidence interval determined using StatXact PROCs for SAS, version 6 (Cytel Statistical Software).

The humoral immune response against *P. falciparum* was assessed as described elsewhere by determining titers of antibody to the circumsporozoite protein. Seropositivity was defined as anti-circumsporozoite protein titers of  $\geq 0.5$  EU/mL. Analyses were performed using SAS software (version 8; SAS) and STATA software (version 9.0; Stata).

## RESULTS

For the safety analysis (surveillance months 0–45), a total of 2022 children aged 1–4 years were recruited and randomized to the RTS,S/AS02A group and the control group (1605 children for cohort 1 and 417 children for cohort 2). A total of 1465 subjects (72.5%; 1142 subjects in cohort 1 and 323 subjects in cohort 2) completed the follow-up to study month 45. For the efficacy analyses (months 2.5–45), including only those participants in cohort 1, a total of 1490 (73.7%) of 2022 children completed the follow-up (figure 2).

Over the 45-month surveillance period analyzed for the intent-to-treat cohort, 639 SAEs classified according to the preferred term in the *Medical Dictionary for Regulatory Activities* [19] were noted in 235 subjects who received the RTS,S/AS02A vaccine, and 770 SAEs were noted in 326 subjects who received the control vaccines (table 1). The pattern of the causes of SAEs observed in this trial is similar to the morbidity background of the area. The most important diseases are malaria, anemia,

**Table 2. Vaccine Efficacy (as Determined by According-to-Protocol Analysis of Follow-up Months 2.5–45) in Cohort 1, by Outcome**

	Control vaccine group (n = 745)			RTS,S/AS02A vaccine group (n = 745)			Vaccine efficacy, % (95% CI)	P
	Events, no.	PYAR, no.	Event rate	Events, no.	PYAR, no.	Event rate		
Fever								
First or only episode								
And parasitemia								
>0 parasite/ $\mu$ L	393	1380.1	0.29	342	1571.3	0.22	27.2 (15.6–27.1)	<.001
>2500 parasites/ $\mu$ L	370	1440.1	0.26	307	1637.4	0.19	30.5 (18.9–40.4)	<.001
>15,000 parasites/ $\mu$ L	334	1520.3	0.22	282	1699.4	0.17	28.2 (15.7–38.9)	<.001
Or history of fever and parasitemia								
>0 parasites/ $\mu$ L	490	1137.0	0.43	421	1378.8	0.31	31.9 (22.2–40.4)	<.001
Several episodes and parasitemia								
>2500 parasites/ $\mu$ L	774	2142.8	0.36	658	2194.3	0.30	25.6 (11.9–37.1)	<.001

**NOTE.** The 1605 participants in cohort 1 were monitored using passive surveillance, to detect clinical episodes of malaria, and safety surveillance. Vaccine efficacy estimates were adjusted by age at baseline, bed net use at baseline, distance from health care facility, and geographic region. CI, confidence interval; PYAR, person-years at risk.

gastroenteritis, and pneumonia. During this period, 62 cases of severe malaria were experienced by 4.6% (95% CI, 3.4%–6.1%) of the study participants who received RTS,S/AS02A. In the control group, there were 83 cases of severe malaria among 7.0% (95% CI, 5.5%–8.8%) of the study participants. Blood transfusions were performed for 58 subjects (2.7% of patients in the group receiving study vaccine and 3.1% of patients in the control group). There were 34 deaths, with 12 (1.2% [95% CI, 0.6%–2.1%]) occurring in the RTS,S/AS02A group and 22 (2.2% [95% CI, 1.4%–3.35%]) occurring in the control group ( $P = .087$ ). Six of these deaths were judged to be associated with malaria: 1 occurred in the RTS,S/AS02A group, and 5 occurred in control group. No SAE or death was considered to be associated with vaccination.

**VE.** In the VE analyses (VE analysis for the according-to-protocol cohort for months 2.5–45 [ATP<sub>{2.5–45}</sub>]), 677 children had first or only clinical episodes that met the primary case definition. Of these, 307 were in the RTS,S/AS02A group and 370 were in the control group, yielding a crude VE estimate of 25.6% (95% CI, 13.4%–36.0%;  $P < .001$ ) and an adjusted VE<sub>(2.5–45)</sub> of 30.5% (95% CI, 18.9%–40.4%;  $P < .001$ ) (figure 3). The VE estimates obtained using several case definitions based on different parasite-density cutoff levels are shown in table 2. The adjusted VE<sub>(2.5–45)</sub> of surveillance, including all clinical episodes, was 25.6% (95% CI, 11.9%–37.1%;  $P < .001$ ).

In the RTS,S/AS02A group ( $n = 745$ ), there were 29 children who had  $\geq 1$  episode of severe malaria, compared with 47 children in the control group ( $n = 745$ ) (VE, 38.3% [95% CI, 3.4%–61.3%];  $P = .045$ ). The number of hospital admissions due to all causes was also lower for the RTS,S/AS02A group than for the control group (175 vs 194 admissions), and the

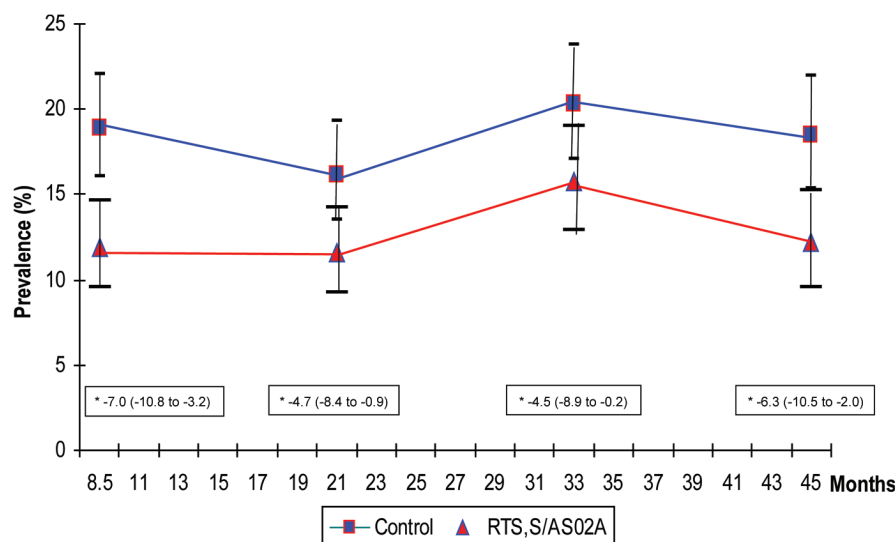
VE was 22.2% (95% CI, –3.8% to 41.7%;  $P = .088$ ). The VE against malaria resulting in hospitalization was 23.0% (95% CI, –1.7% to 41.9%;  $P = .078$ ).

Analysis of VE over different follow-up periods showed a VE of 16.8% over months 21–33 (95% CI, –2.5% to 32.4%;  $P = .084$ ) and a VE of 11.8% over months 33–45 (95% CI, –20.1% to 35.2%;  $P = .426$ ). There is a trend toward lower estimates of efficacy over the latter follow-up periods, but the proportionality of the hazard assumption did not find evidence of waning efficacy either by graphical inspection of the plot of log [–log(survival time)] with log of the survival time, the time-dependent Cox models, or the test based on the Schoenfeld residuals ( $P = .210$ ).

#### **Anti-circumsporozoite protein response and parasitemia.**

Anti-circumsporozoite protein antibody levels were still  $\sim 30$ -fold higher than prevaccination levels in cohort 1 at month 45 in the RTS,S/AS02A group, with a geometric mean titer (GMT) of 8.9 (95% CI, 7.8–10.1), whereas most of the children in the control group had a GMT of 0.3 (95% CI, 0.3–0.3). At least 96% of subjects in the RTS,S/AS02A group were seropositive for anti-circumsporozoite protein antibodies at month 45.

The prevalence of asexual-stage parasites was lower in the RTS,S/AS02A group than in the control group, in the yearly cross-sectional surveys that were performed (figure 4). At study month 33, the prevalence of *P. falciparum* asexual-stage parasitemia was 22% lower in the RTS,S/AS02A group (93 [15.8%] of 590 patients) than in the control group (121 [20.3%] of 596 patients;  $P = .049$ ). At study month 45, prevalence was 34% lower in the RTS,S group (66 [12.2%] of 541 patients) than in the control group (101 [18.5%] of 547 patients) ( $P = .004$ ). Among children bearing asexual-stage *P. falciparum* parasites



**Figure 4.** Prevalence (95% confidence interval) of *Plasmodium falciparum* asexual-stage parasitemia at the different cross-sectional surveys. \*Difference (95% confidence interval) in the proportion of positives between the RTS,S/AS02A group and the control group.

during the cross-sectional surveys, parasite densities were similar in the 2 groups and at both surveys conducted at months 33 and 45 (geometric mean density, 1878 vs 1621 per  $\mu\text{L}$  [ $P = .467$ ] and 594 vs 1057 per  $\mu\text{L}$  [ $P = .065$ ], respectively) (figure 4).

## DISCUSSION

The present study reports what is, to our knowledge, the first long-term follow-up of a pediatric malaria vaccine trial in Africa. Over a 45-month period, the candidate vaccine had an acceptable safety profile with significantly less SAEs and a trend toward a reduced mortality rate among individuals in the RTS,S/AS02A group.

Previous reports confirmed efficacy during an initial 6-month follow-up as well as sustained protection up to 21 months of follow-up. Analysis up to 45 months allows us to exclude the theoretical risk that partial protection with this vaccine could have impaired acquisition of natural immunity and that subsequent loss of vaccine-induced protection could be followed by a rebound in the risk of clinical malaria among previously protected children.

It is challenging to assess the duration of protection against a communicable disease when repeated infections and clinical episodes are required to slowly build up naturally acquired immunity, and when the risk of malaria consequently is age dependent. Indeed, over the past 2 years, the incidence of clinical malaria in the control group decreased from 0.37 episodes/person-years at risk (during follow-up from months 21 to 33) to 0.15 episodes/person-years at risk (during follow-up from months 33 to 45). Analysis of efficacy broken down into 12-

month periods yields estimates that show a tendency toward decreasing efficacy from 30% to 11%, with overlapping confidence intervals that are wider at the end indicating less precision on the estimate at the end of the study. The statistical method used to evaluate the proportionality of the hazard assumption showed no evidence of waning efficacy, but because the study was not designed to have sufficient power to evaluate this, it could reflect a lack of power to detect it.

On the other hand, the prevalence of parasites at the end of the 45-month follow-up was significantly lower in the vaccine group than in the control group. Given that the prevalence of *P. falciparum* asexual-stage parasitemia must reflect the recent risk of infection, we interpret this finding as a strong indication that significant efficacy remains at the end of the 45-month follow-up.

VE against clinical malaria and against severe malaria over the entire follow-up was 30.5% (95% CI, 18.9%–40.4%) and 38.3% (95% CI, 3.4%–61.3%), respectively. VE against all clinical episodes was 25.6% (95% CI, 11.9%–37.1%;  $P < .001$ ). In other words, immunization with RTS,S/AS02A reduced the burden of malaria during this period by approximately one-quarter.

These exciting results confirm the potential of developing malaria vaccines that may influence relevant end points of clinical and public health and that may consequently reduce the unacceptable burden of malaria in African children. Together with recently reported data showing a favorable safety profile and a proof-of-concept efficacy of 65% in reducing the risk of new infections when vaccine is administered to young infants at 10, 14, and 18 weeks of age or coadministered with routine

EPI vaccine [12, 20], these results strengthen the rationale for advancing toward a phase 3 trial aiming to register RTS,S/AS as the first malaria vaccine.

## Acknowledgments

A complex trial like this one would not have been possible without the contribution of a large number of people. We would first like to thank the children and parents who participated in the study, as well as the entire Manhica community, for their continuous support of this and all other activities that Centro de Investigação em Saúde de Manhica (CISM) has undertaken since 1996. We would also like to thank all CISM staff.

GlaxoSmithKline (GSK) is grateful to the staff of the Malaria Project team at GSK, in particular Christine Swysen, Bruno Knez, Alex Owusu, Alfred Tiono, Bart Spiessens, Laurence Vigneron, and Sabine Corachan.

PATH–Malaria Vaccine Initiative thanks Regina Rabinovich, Director, Infectious Diseases Program, Bill & Melinda Gates Foundation, for her significant contributions to the project.

Esperança Sevene was the local safety monitor for this trial. She provided invaluable and continuous support and guidance. Malcolm Molyneux was the chairman of the data and safety monitoring board. His advice and support were greatly appreciated by the investigators. Special thanks to the district, provincial, and national health authorities for their support.

**Financial support.** GlaxoSmithKline Biologicals (GSK) and Centro de Investigação em Saúde de Manhica (CISM) both received financial support from PATH–Malaria Vaccine Initiative (MVI) to conduct the work described in this article. The initial support to CISM by PATH–MVI was passed through GSK for administrative purposes. CISM is supported by the members of the collaborative programme (Ministry of Health [Mozambique], Universidade Eduardo Mondlane [Mozambique], and Hospital Clinic [Universidad de Barcelona, Spain]), and core funding is provided by the Spanish Agency for International Cooperation and Development (AECID–Ministry of Foreign Affairs and Cooperation, Madrid, Spain).

## References

1. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* **2005**; 434:214–7.
2. Breman JG. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg* **2001**; 64:1–11.
3. Bojang KA, Milligan PJM, Pinder M, et al. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet* **2001**; 358: 1927–34.
4. Stewart VA, McGrath SM, Walsh DS, et al. Pre-clinical evaluation of new adjuvant formulations to improve the immunogenicity of the malaria vaccine RTS,S/AS02A. *Vaccine* **2006**; 24:6483–92.
5. Stoute JA, Slaoui M, Heppner DG, et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. *N Engl J Med* **1997**; 336:86–91.
6. Bojang KA, Oludude F, Pinder M, et al. Safety and immunogenicity of RTS,S/AS02A candidate malaria vaccine in Gambian children. *Vaccine* **2005**; 23:4148–57.
7. Kester KE, McKinney DA, Tornieporth N, et al. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. *J Infect Dis* **2001**; 183:640–7.
8. Doherty JF, Pinder M, Tornieporth N, et al. A phase I safety and immunogenicity trial with the candidate malaria vaccine RTS,S/SBAS2 in semi-immune adults in The Gambia. *Am J Trop Med Hyg* **1999**; 61: 865–8.
9. Macete E, Aponte JJ, Guinovart C, et al. Safety and immunogenicity of the RTS,S/AS02A candidate malaria vaccine in children aged 1–4 in Mozambique. *Trop Med Int Health* **2007**; 12:37–46.
10. Alonso PL, Sacarlal J, Aponte JJ, et al. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. *Lancet* **2004**; 364:1411–20.
11. Alonso PL, Sacarlal J, Aponte JJ, et al. Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. *Lancet* **2005**; 366:2012–8.
12. Aponte JJ, Aide P, Renom M, et al. Safety of the RTS,S/AS02D candidate malaria vaccine in infants living in a highly endemic area of Mozambique: a double blind randomised controlled phase I/IIb trial. *Lancet* **2007**; 370:1543–51.
13. Loscertales MP, Roca A, Ventura PJ, et al. Epidemiology and clinical presentation of respiratory syncytial virus infection in a rural area of southern Mozambique. *Pediatr Infect Dis J* **2002**; 21:148–55.
14. Alonso PL, Saute F, Aponte JJ, et al.; Manhica Demographic Surveillance System (DSS), Mozambique. In: Population and health in developing countries. Vol 1. Ottawa, Cairo, Dakar, Montevideo, Nairobi, New Dehli, Singapore: International Development Research Center, **2002**:189–95.
15. Sacarlal J, Aponte JJ, Aide P, et al. Safety of the RTS,S/AS02A malaria vaccine in Mozambican children during a phase IIb trial. *Vaccine* **2008**; 26: 174–84.
16. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. *Trans R Soc Trop Med Hyg* **2000**; 94:S1–90.
17. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model (statistics for biology and health). New York: Springer, **2000**.
18. Hess KR. Graphical methods for assessing violations of the proportional hazards assumption in Cox regression. *Stat Med* **1995**; 14:1707–23.
19. The medical dictionary for regulatory activities. **2007**.
20. Abdulla S, Oberholzer R, Juma O, et al. Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. *N Engl J Med* **2008**; 359: 2533–44.