MAJOR ARTICLE

Evaluation of the Safety and Immunogenicity of the RTS,S/AS01$_E$ Malaria Candidate Vaccine When Integrated in the Expanded Program of Immunization

Selidji T. Agnandji,[1,2] Kwaku Poku Asante,[3,4] John Lyimo,[5] Johan Vekemans,[6] Solange S. Soulanoudjingar,[1,2] Ruth Owusu,[3] Mwanajaa Shomari,[5] Amanda Leach,[6] Jose Fernandes,[1,2] David Dosoo,[3] Maria Chikawe,[5] Saadou Issifou,[1,2] Kingsley Osei-Kwakye,[3] Marc Lievens,[6] Maria Paricek,[1,2] Stephen Apanga,[3] Grace Mwangoka,[5] Blaise Okissi,[1,2] Evans Kwara,[3] Rose Minja,[5] Jorn Lange,[1,2] Owusu Boahen,[3] Kingsley Kayan,[3] George Adjei,[3] Daniel Chandramohan,[3,4] Erik Jongert,[6] Marie-Ange Demoitié,[6] Marie-Claude Dubois,[6] Terrel Carter,[7] Preeti Vansadia,[7] Tonya Villafana,[7] Marla Sillman,[7] Barbara Savarese,[7] Didier Lapierre,[6] William Ripley Ballou,[6] Brian Greenwood,[3,4] Marcel Tanner,[5,8] Joe Cohen,[6] Peter G. Kremsner,[1,2] Bertrand Lell,[1,2] Seth Owusu-Agyei,[3,4] and Salim Abdulla[5]

[1]Albert Schweitzer Hospital, Medical Research Unit Lambaréné, Lambaréné, Gabon; [2]Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany; [3]Kintampo Health Research Centre, Ghana Health Service, Kintampo, Ghana; [4]London School of Hygiene and Tropical Medicine, London, United Kingdom; [5]Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo District Hospital, Bagamoyo, Tanzania; [6]GlaxoSmithKline Biologicals, Rixensart, Belgium; [7]Program for Appropriate Technology in Health Malaria Vaccine Initiative, Bethesda, Maryland; [8]Swiss Tropical Institute, University of Basel, Basel, Switzerland

Background. The RTS,S/AS01$_E$ malaria candidate vaccine is being developed for immunization of African infants through the Expanded Program of Immunization (EPI).

Methods. This phase 2, randomized, open, controlled trial conducted in Ghana, Tanzania, and Gabon evaluated the safety and immunogenicity of RTS,S/AS01$_E$ when coadministered with EPI vaccines. Five hundred eleven infants were randomized to receive RTS,S/AS01$_E$ at 0, 1, and 2 months (in 3 doses with diphtheria, tetanus, and whole-cell pertussis conjugate [DTPw]; hepatitis B [HepB]; *Haemophilus influenzae* type b [Hib]; and oral polio vaccine [OPV]), RTS,S/AS01$_E$ at 0, 1, and 7 months (2 doses with DTPwHepB/Hib+OPV and 1 dose with measles and yellow fever), or EPI vaccines only.

Results. The occurrences of serious adverse events were balanced across groups; none were vaccine-related. One child from the control group died. Mild to moderate fever and diaper dermatitis occurred more frequently in the RTS,S/AS01$_E$ coadministration groups. RTS,S/AS01$_E$ generated high anti–circumsporozoite protein and anti–hepatitis B surface antigen antibody levels. Regarding EPI vaccine responses upon coadministration when considering both immunization schedules, despite a tendency toward lower geometric mean titers to some EPI antigens, predefined noninferiority criteria were met for all EPI antigens except for polio 3 when EPI vaccines were given with RTS,S/AS01$_E$ at 0, 1, and 2 months. However, when antibody levels at screening were taken into account, the rates of response to polio 3 antigens were comparable between groups.

Conclusion. RTS,S/AS01$_E$ integrated in the EPI showed a favorable safety and immunogenicity evaluation.

Trial registration. ClinicalTrials.gov identifier: NCT00436007. GlaxoSmithKline study ID number: 106369 (Malaria-050).

The development of a malaria vaccine has been identified as a key component of future integrated malaria control programs and an important step toward sustainable elimination of malaria in Africa. Improved control of malaria would have significant benefits in health and for the economy of sub-Saharan Africa [1–7].

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Reprints or correspondence: Selidji T. Agnandji, Albert Schweitzer Hospital, Medical Research Unit Lambaréné, Gabon (agnandjis@yahoo.fr).

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Potential conflicts of interest: J.V., A.L., M.L., E.J., M.-A.D., M.-C.D., D.L., W.R.B., and J.C. are employees of GlaxoSmithKline Biologicals. J.V., A.L., M.-C.D., W.R.B., and J.C. own shares in GlaxoSmithKline. J.C. and W.R.B. were listed as inventors of patented malaria vaccines, including RTS,S. T.C., P.V., T.V., M.S., and B.S. are employees of Malaria Vaccine Initiative, which supports the development and testing of several malaria vaccines. All other authors report no potential conflicts.

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The RTS,S/AS01 vaccine is being developed for the routine immunization of infants and children living in areas where malaria is endemic as part of the Expanded Program of Immunization (EPI). The vaccine antigen RTS,S consists of sequences of the *Plasmodium falciparum* circumsporozoite (CS) protein and the hepatitis B surface antigen (HBsAg). The GlaxoSmithKline proprietary adjuvant system AS01 contains the MPL and QS21 immunostimulants and liposomes. Past trials of RTS,S have been conducted with the closely related adjuvant system AS02, which contains the same immunostimulants MPL and QS21 and an oil-in-water emulsion.

Phase 2 trials of RTS,S/AS02 have demonstrated a favorable safety profile in infants when the vaccine was given separately or in coadministration with EPI vaccines [8, 9]. Noninferiority of antibody responses against coadministered diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b (Hib) vaccines has been demonstrated [9]. RTS,S/AS02 induced 65% protection against *P. falciparum* infection over 6 months when coadministered with EPI vaccines, despite lower anti-CS titers than those induced when administration was staggered 2 weeks apart from the administration of EPI vaccines.

Both in adults and subsequently in children, the RTS,S/AS01 formulation showed improved efficacy and immunogenicity and an equally favorable safety profile as compared with RTS,S/AS02, which supports the selection of RTS,S/AS01 for further development for children and infants [10–12]. RTS,S/AS01$_E$ is the pediatric formulation of RTS,S/AS01. In subjects aged 5–17 months, the measured vaccine efficacy of RTS,S/AS01$_E$ against clinical malaria was 53%, over a mean 8-month follow-up period [13].

The delivery of a new malaria vaccine through the EPI would be the most effective means of achieving rapid high coverage. We studied the safety and immunogenicity of RTS,S/AS01$_E$ administered to infants aged 6–10 weeks at the time of the first administration, when coadministered with the diphtheria, tetanus, and whole-cell pertussis conjugate (DTPw) vaccine, hepatitis B (HepB) vaccine, Hib vaccine, oral polio vaccine (OPV), measles vaccine, and yellow fever vaccine routinely used in the EPI in sub-Saharan Africa. Two RTS,S/AS01$_E$ regimens (vaccination at 0, 1, and 2 months and vaccination at 0, 1, and 7 months) were studied; both constitute alternative options for integration into the EPI.

METHODS

Study design. The study was a phase 2, randomized, controlled, open study and was prospectively registered at ClinicalTrials.gov. Approval was obtained from the Ifakara Health Research and Development Centre and the National Review Committee of the National Institution for Medical Research in Tanzania, the Comité d'Ethique Régional Indépendant de Lambaréné in Gabon, the Kintampo Health Research Center Scientific Review Committee/Institutional Ethics Committee and the Ghana Health Service National Ethical Review Committee in Ghana, the London School of Hygiene and Tropical Medicine Ethics Committee in the United Kingdom, the Swiss Tropical Institute Committee in Switzerland, and the Western Institutional Review Board in the United States.

The independent data monitoring committee appointed to oversee the RTS,S pediatric development program reviewed the ethical, quality, and safety aspects of the study conduct. The study was conducted in accordance with the Helsinki Declaration of 1964 (revised in 1996) and according to Good Clinical Practice guidelines.

GlaxoSmithKline Biologicals was the study sponsor. The Program for Appropriate Technology in Health Malaria Vaccine Initiative cofunded this trial and was involved in all aspects of the study design.

Study research centers. The study was conducted at 3 clinical research centers: Kintampo Health Research Center, Kintampo, Ghana; Ifakara Health Research and Development Centre, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania; and the Albert Schweitzer Hospital, Medical Research Unit Lambaréné, Lambaréné, Gabon. The intensity of malaria transmission in all 3 study sites is intense and perennial [11, 14–17].

Study participants. For the recruitment of study participants, lists of potentially eligible infants were generated following community-based information programs in the Bagamoyo and Lambaréné study areas. In Kintampo, the monitoring of births as part of the research center's demographic surveillance system was used. Healthy male and female infants aged 6–10 weeks at the time of the first vaccine dose who had received 1 previous dose of OPV and bacille Calmette-Guérin (BCG) were eligible for enrollment. Written informed consent was obtained from each child's parent or guardian before study procedures were initiated. For nonliterate parents, consent was documented using a thumbprint and a signature by a literate witness.

Randomization and vaccination. Eligible subjects were randomized (1:1:1) to 1 of 3 groups (Table 1). The RTS,S/AS01$_E$ (0, 1, 2) group received RTS,S/AS01$_E$ at 0, 1, and 2 months—3 doses in coadministration with DTPwHepB/Hib+OPV. The RTS,S/AS01$_E$ (0, 1, 7) group received RTS,S/AS01$_E$ at 0, 1, and 7 months—doses 1 and 2 in coadministration with DTPwHepB/Hib+OPV and dose 3 in coadministration with measles and yellow fever vaccines. The control group received EPI vaccines only. Yellow fever vaccine was not administered to infants from Tanzania because it is not included in the EPI vaccination schedule in Tanzania.

Study vaccines. RTS,S/AS01$_E$, DTPwHepB/Hib (Tritanrix HepB and Hiberix; GlaxoSmithKline Biologicals), measles vaccine (Rouvax; Aventis Pasteur; some subjects from Gabon received the local EPI measles vaccine from the Serum Institute

Table 1. Outline of Study Design to Test Safety and Immunogenicity of RTS,S/AS01$_E$ Malaria Candidate Vaccine Integrated in the Expanded Program of Immunization

Study group, vaccinations received and blood sampling plan	Study month									
	−1 (screening)	0	1	2	3	4	5	6	7	8
Control (N = 171)										
Vaccines										
DTPwHepB/Hib+OPV		X	X	X						
Measles, yellow fever[a]									X	
Antibody level determination										
CS, HBsAg	X				X				X	X
BPT	X				X					
DTPw, Hib, polio	X[b]				X					
Measles, yellow fever[a]									X	X
Blood sample tested for safety	X	X[c]			X					X
RTS,S/AS01$_E$ (0, 1, 2) (N = 170)										
Vaccines										
RTS,S/AS01$_E$		X	X	X						
DTPwHepB/Hib+OPV		X	X	X						
Measles, yellow fever[a]									X	
Antibody level determination										
CS, HBsAg	X			X	X				X	
BPT	X				X					
DTPw, Hib, polio	X[b]				X					
Measles, yellow fever[a]										
Blood sample tested for safety	X	X[c]			X					
RTS,S/AS01$_E$ (0, 1, 7) (N = 170)										
Vaccines										
RTS,S/AS01$_E$		X	X						X	
DTPwHepB/Hib+OPV		X	X	X						
Measles, yellow fever[a]									X	
Antibody level determination										
CS, HBsAg	X				X				X	X
BPT	X				X					
DTPw, Hib, polio	X[b]				X					
Measles, yellow fever[a]									X	X
Blood sample tested for safety	X	X[c]								X

NOTE. BPT, *Bordetella pertussis* toxin; CS, circumsporozoite protein of *Plasmodium falciparum*; DTPw, diphtheria, tetanus, and pertussis (whole cell) conjugate; HBsAg, hepatitis B surface antigen; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; OPV, oral polio vaccine.

[a] Excluding infants from Tanzania.

[b] As part of a post hoc analysis, anti-polio antibodies were measured at screening.

[c] Safety blood samples were evaluated at day 6 after the first vaccine dose.

of India Limited), and yellow fever vaccine (Stamaril; Aventis Pasteur) were administered intramuscularly. OPV (Polio Sabin; GlaxoSmithKline Biologicals) was administered orally. Vaccinees were observed for 60 min after each vaccination.

Assessment of reactogenicity and safety. Solicited reactogenicity data on adverse events (AEs; pain, swelling, drowsiness, fever, irritability, and loss of appetite) were collected for 7 d following each dose, and reports of unsolicited nonserious AEs were collected for 30 d following each dose. Grade 3 (severe) events were defined as follows: pain that caused the infant to cry when the limb was moved and/or was spontaneous, swelling of >20 mm in diameter, fever with an axillary temperature of ⩾37.5°C (especially of >39°C), irritability (crying that could not be comforted) that prevented normal activity, drowsiness that prevented normal activity, and loss of appetite (not eating at all).

Serious AEs (SAEs) were recorded throughout the study period (months 0–8). All seizures occurring within 30 d after vaccination were reported as SAEs, and those occurring within 7 d were reported according to the Brighton Collaboration guidelines [18].

Time points for the measurement of levels hematological function (hemoglobin, platelets, and white blood cells), renal function (creatinine), and hepatic function (alanine amino-

transferase) are detailed in Table 1. Clinically significant abnormal laboratory findings were reported as AEs or SAEs. Grade 3 abnormalities were predefined as follows: hemoglobin level, <5.0 g/dL; total white blood cell count, $<1.4 \times 10^3$ cells/μL; platelet count, $<25 \times 10^3$ cells/μL; alanine aminotransferase level, >5.1 times the upper limit of the reference range; creatinine level, >3.1 times the upper limit of the reference range.

Assessment of immunogenicity. Time points for measurement of levels of antibodies against CS, HBsAg, diphtheria toxin, tetanus toxin, polyribosyl ribitol phosphate (PRP) for Hib, *Bordetella pertussis* toxin (BPT), polio 1, polio 2, polio 3, measles, and yellow fever antigens are detailed in Table 1.

The levels of immunoglobulin G antibodies to CS were measured by a R32LR antigen-based enzyme-linked immunosorbent assay (ELISA) with a detection cutoff of 0.5 EU/mL, as described elsewhere [19]. Antibody levels were measured using an in-house ELISA for anti-HBsAg (seroprotective cutoff, 10 mIU/mL) [12], anti-diphtheria (seroprotective cutoff, 0.1 IU/mL), anti-PRP (Hib; seroprotective cutoff, 0.15 μg/mL), and anti-tetanus (seroprotective cutoff, 0.1 IU/mL). Antibody levels were measured for anti-BPT by use of a commercial immunoglobulin G ELISA (Anilabsystems; seropositivity cutoff, >15 EI.U/mL), for anti-measles immunoglobulin G by use of a commercial ELISA (Enzygnost; Dade Behring; seroconversion cutoff, 150 mIU/mL), for titers of anti–polio 1, anti–polio 2, and anti–polio 3 by use of a standard poliovirus microneutralization assay (median effective dose protective serum dilution factor, 8), and for anti–yellow fever by use of a yellow fever plaque reduction neutralization test (protective serum dilution factor, 10).

Statistical methods. Statistical analyses were conducted using SAS (version 8; SAS Institute). The trial sample size was calculated to give 90% power to detect a 2.5-fold difference (2-sided; 5% significance) in the rate of a SAE occurring with a frequency of 10% in the control group compared to each of the experimental regimens. For secondary immunogenicity endpoints, the trial had >95% power to demonstrate noninferiority for all antigens except measles antigen, for which power was 82%, based on historical response rates.

Safety was analyzed on the total vaccinated cohort, which included all subjects with at least 1 documented study vaccine administration. The primary endpoint was the occurrence of SAEs from months 0–8. The proportion of subjects with a SAE, as classified by the preferred term in the Medical Dictionary for Regulatory Activities (MedDRA), was tabulated with exact 95% confidence intervals (CIs). Summaries were tabulated for the incidence, intensity, and relationship of solicited symptoms and unsolicited AEs, as classified by MedDRA preferred terms. According to protocol, all solicited local injection site symptoms were considered to be causally related to vaccination. Frequency distributions of biochemical and hematological parameters by severity grades were tabulated.

The primary analysis of immunogenicity was performed on the According to Protocol cohort, which included subjects who met all eligibility criteria, complied with the study procedures, and had no elimination criteria. Seropositivity, seroprotection, or seroconversion rates were defined with 95% CIs. Antibody titers were summarized by geometric mean titers (GMTs) with 95% CIs for all antigens.

For the analysis of noninferiority, the differences in anti-HBsAg, anti-diphtheria, anti-tetanus, anti-PRP, anti–polio 1, anti–polio 2, and anti–polio 3 seroprotection rates at month 3 and those in anti-measles and anti–yellow fever seroconversion rates at month 8 between the control group and RTS,S/AS01$_E$ groups were calculated with 95% CIs (standardized asymptotic) around this difference. If the upper limit of the 2-sided 95% CI was <10%, then the noninferiority of the RTS,S/AS01$_E$ groups compared with the control group was considered to be demonstrated. For anti-BPT, for which there is no demonstrated correlate of protection, the 95% CI (analysis of variance model; pooled variance) on the GMT ratio (GMT of the control group divided by that of the RTS,S/AS01$_E$ group) was calculated. If the upper limit of this 95% CI was <1.5, then the noninferiority of the RTS,S/AS01$_E$ groups compared with the control group was considered to be demonstrated. As a post hoc analysis, polio vaccine responses were investigated when taking into account polio antibody titers at screening. A polio vaccine response was defined as the appearance of antibodies at month 3 (titer, $\geqslant$8 EU/mL) in initially seronegative subjects or a 2-fold increase in postvaccination antibody titers over prevaccination titers in initially seropositive subjects.

RESULTS

Subject cohort. Figure 1 summarizes subject participation during the course of the study. Age, sex, and weight were balanced between groups. The mean age of subjects at baseline was 7.0 weeks (standard deviation, 1.0 weeks), the mean weight at baseline was 4.9 kg (standard deviation, 0.6 kg), and 51% of the subjects were male. All participants had received a first dose of OPV and BCG vaccine in the neonatal period.

Safety outcome primary endpoint. From the time of the first vaccination until month 8, the proportion of subjects with a SAE was similar in all groups: 22.9% in the RTS,S/AS01$_E$ (0, 1, 2) group, 18.2% in the RTS,S/AS01$_E$ (0, 1, 7) group, and 21.1% in the control group (Table 2). No SAE occurred with a clinically concerning higher incidence in either RTS,S/AS01$_E$ groups compared with the control group.

No seizures occurred within 7 d after vaccination. Three seizures were reported within 30 d after vaccination, 2 in subjects from the RTS,S/AS01$_E$ (0, 1, 7) group and 1 in a subject from the control group.

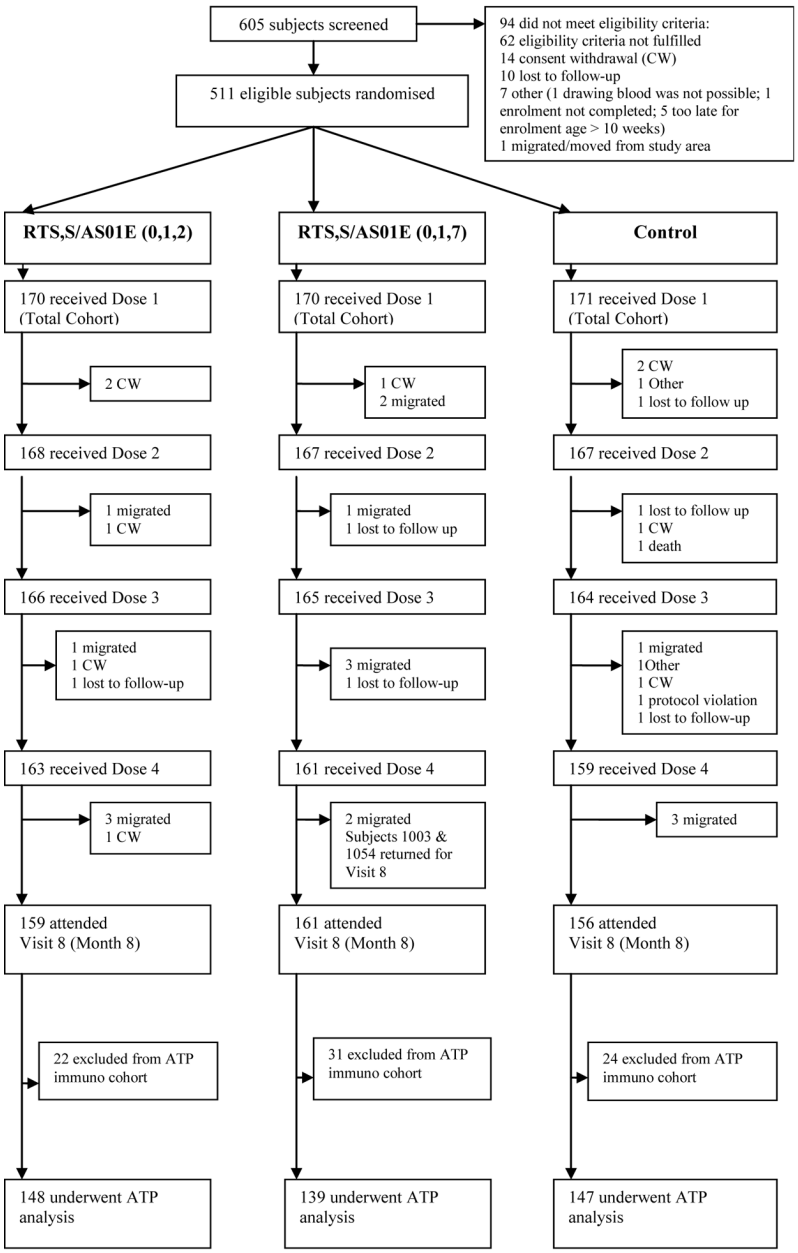

Figure 1. Summary of subject participation in a phase 2, randomized, open, controlled trial conducted in Ghana, Tanzania, and Gabon to evaluate the safety and immunogenicity of the RTS,S/AS01$_E$ malaria candidate vaccine coadministered with Expanded Program of Immunization vaccines. Subjects in the RTS,S/AS01$_E$ (0, 1, 2) group were vaccinated with RTS,S/AS01$_E$ and DTPwHepB/Hib+OPV (diphtheria, tetanus, and whole-cell pertussis conjugate; hepatitis B; *Haemophilus influenzae* type b; and oral polio vaccine) at vaccine visits 1, 2, and 3 (doses 1, 2, and 3) and with measles and yellow fever vaccines at vaccine visit 4 (dose 4). Subjects in the RTS,S/AS01$_E$ (0, 1, 7) group were vaccinated with RTS,S/AS01$_E$ and DTPwHepB/Hib+OPV at vaccine visits 1 and 2 (doses 1 and 2), with DTPwHepB/Hib+OPV at vaccine visit 3 (dose 3), and with RTS,S/AS01$_E$ and measles and yellow fever vaccines at vaccine visit 4 (dose 4). Subjects in the control group were vaccinated with DTPwHepB/Hib+OPV at vaccine visits 1, 2, and 3 (doses 1, 2, and 3) and with measles and yellow fever vaccines at vaccine visit 4 (dose 4).

No SAE was considered by the investigator to be related to vaccination. One study participant in the control group died because of pneumonia and severe *P. falciparum* malaria with severe anemia.

Solicited AEs. The occurrence of solicited events following vaccination is summarized in Table 3. No local solicited injection site pain of grade 3 occurred. Grade 3 swelling was rare, occurring after $\leqslant$0.6% of doses of any vaccine in each study group.

Fever was reported more frequently in participants who received RTS,S/AS01$_E$ in combination with DTPwHepB/Hib+OPV compared with participants who received DTPwHepB/Hib+OPV

Table 2. Occurrence of Unsolicited Adverse Events in the Total Vaccinated Cohort

MedDRA preferred term	RTS,S/AS01$_E$ (0, 1, 2) group (N = 170)		RTS,S/AS01$_E$ (0, 1, 7) group (N = 170)		Control group (N = 171)	
	No. of subjects[a]	% (95% CI)	No. of subjects[a]	% (95% CI)	No. of subjects[a]	% (95% CI)
	Serious adverse events occurring in >2% of subjects in any vaccine group (whole follow-up)					
At least 1 serious adverse event	39	22.9 (16.9–30.0)	31	18.2 (12.7–24.9)	36	21.1 (15.2–27.9)
Anemia	6	3.5 (1.3–7.5)	10	5.9 (2.9–10.6)	14	8.2 (4.5–13.4)
Febrile convulsion	1	0.6 (0.0–3.2)	4	2.4 (0.6–5.9)	0	0.0 (0.0–2.1)
Gastroenteritis	19	11.2 (6.9–16.9)	11	6.5 (3.3–11.3)	14	8.2 (4.5–13.4)
Impetigo	3	1.8 (0.4–5.1)	1	0.6 (0.0–3.2)	4	2.3 (0.6–5.9)
Plasmodium falciparum infection	5	2.9 (1.0–6.7)	10	5.9 (2.9–10.6)	16	9.4 (5.4–14.7)
Pneumonia	11	6.5 (3.3–11.3)	10	5.9 (2.9–10.6)	10	5.8 (2.8–10.5)
Sepsis	2	1.2 (0.1–4.2)	2	1.2 (0.1–4.2)	4	2.3 (0.6–5.9)
Upper respiratory tract infection	4	2.4 (0.6–5.9)	5	2.9 (1.0–6.7)	6	3.5 (1.3–7.5)
	Nonserious adverse events occurring in >5% of subjects in any vaccine group (30 days after vaccination)					
At least 1 adverse event	160	94.1 (89.4–97.1)	161	94.7 (90.2–97.6)	164	95.9 (91.7–98.3)
Anemia	11	6.5 (3.3–11.3)	19	11.2 (6.9–16.9)	11	6.4 (3.3–11.2)
Bronchitis	17	10.0 (5.9–15.5)	17	10.0 (5.9–15.5)	21	12.3 (7.8–18.2)
Conjunctivitis	16	9.4 (5.5–14.8)	21	12.4 (7.8–18.3)	19	11.1 (6.8–11.2)
Cough	21	12.4 (7.8–18.3)	30	17.6 (12.2–24.2)	24	14.0 (9.2–20.2)
Dermatitis diaper	8	4.7 (2.1–9.1)	9	5.3 (2.–9.8)	0	0.0 (0.0–2.1)
Diarrhoea	21	12.4 (7.8–18.3)	24	14.1 (9.3–20.3)	24	14.0 (9.2–20.2)
Enteritis	8	4.7 (2.1–9.1)	12	7.1 (3.7–12.0)	16	9.4 (5.4–14.7)
Gastroenteritis	29	17.1 (11.7–23.6)	25	14.7 (9.7–20.9)	32	18.7 (13.2–25.4)
Impetigo	7	4.1 (1.7–8.3)	4	2.4 (0.6–5.9)	14	8.2 (4.5–13.4)
Induration	26	15.3 (10.2–21.6)	28	16.5 (11.2–22.9)	29	17.0 (11.7–23.4)
Nasopharyngitis	53	31.2 (24.3–38.7)	62	36.5 (29.2–44.2)	71	41.5 (34.0–49.3)
Otitis media	10	5.9 (2.9–10.6)	8	4.7 (2.1–9.1)	8	4.7 (2.0–9.0)
P. falciparum infection	9	5.3 (2.4–9.8)	11	6.5 (3.3–11.3)	14	8.2 (4.5–13.4)
Pneumonia	19	11.2 (6.9–16.9)	11	6.5 (3.3–11.3)	9	5.3 (2.4–9.8)
Rhinitis	16	9.4 (5.5–14.8)	21	12.4 (7.8–18.3)	21	12.3 (7.8–18.2)
Rhinorrhoea	19	11.2 (6.9–16.9)	19	11.2 (6.9–16.9)	23	13.5 (8.7–19.5)
Skin infection	6	3.5 (1.3–7.5)	8	4.7 (2.1–9.1)	13	7.6 (4.1–12.6)
Staphylococcal skin infection	4	2.4 (0.6–5.9)	10	5.9 (2.9–10.6)	5	2.9 (1.0–6.7)
Upper respiratory tract infection	66	38.8 (31.5–46.6)	66	38.8 (31.5–46.6)	65	38.0 (30.7–45.7)

NOTE. Subjects who received at least 1 vaccine dose were included in the analysis. CI, confidence interval (exact); MedDRA, Medical Dictionary for Regulatory Activities.

[a] Number of subjects for whom the symptom was reported at least once.

alone and in participants who received RTS,S/AS01$_E$ in combination with measles and yellow fever vaccines compared with participants who received measles and yellow fever vaccines alone. Grade 3 fever was rare and balanced across vaccine groups. No grade 3 drowsiness or loss of appetite was reported.

Unsolicited AEs. Unsolicited AEs were reported in similar proportions of subjects in the RTS,S/AS01$_E$ (0, 1, 2), RTS,S/AS01$_E$ (0, 1, 7), and control groups (94.1%, 94.7%, and 95.9%, respectively) (Table 2). Nasopharyngitis and upper respiratory tract infection were the most frequently reported unsolicited symptoms, occurring with a similar incidence in all study groups. Diaper dermatitis occurred more frequently in RTS,S/AS01$_E$ recipients than in control participants (8 subjects in the RTS,S/AS01$_E$ [0, 1, 2] group, 9 subjects in the RTS,S/AS01$_E$ [0, 1, 7] group, and 0 subjects in the control group).

Unsolicited AEs with a causal relationship to vaccination were reported with similar frequencies in all study groups (17.1% in the RTS,S/AS01$_E$ [0, 1, 2] group, 18.2% in the RTS,S/AS01$_E$ [0, 1, 7] group, and 19.9% in the control group), predominantly because of reports of induration from 1 study cen-

Table 3. Incidence of Solicited Adverse Events within 7 Days of Receiving the Study Vaccines among Subjects in the Total Vaccinated Cohort

Vaccination visit, symptom, vaccine[a]	RTS,S/AS01$_E$ (0, 1, 2) group			RTS,S/AS01$_E$ (0, 1, 7) group			Control group		
	No. of administered doses	No. of doses followed by symptom	% (95% CI)	No. of administered doses	No. of doses followed by symptom	% (95% CI)	No. of administered doses	No. of doses followed by symptom	% (95% CI)
After vaccination visits 1, 2, and 3									
Pain									
RTS,S/AS01$_E$	504	259	51.4 (46.9–55.8)	497	232	46.7 (42.2–51.2)	…	…	…
DTPwHepB/Hib	504	279	55.4 (50.9–59.8)	502	279	55.6 (51.1–60.0)	502	282	56.2 (51.7–60.6)
Swelling									
RTS,S/AS01$_E$									
All	504	30	6.0 (4.1–8.4)	497	54	10.9 (8.3–13.9)	…	…	…
>20 mm	504	1	0.2 (0.0–1.1)	497	1	0.2 (0.0–1.1)	…	…	…
DTPwHepB/Hib									
All	504	61	12.1 (9.4–15.3)	502	106	21.1 (17.6–25.0)	502	99	19.7 (16.3–23.5)
>20 mm	504	1	0.2 (0.0–1.1)	502	1	0.2 (0.0–1.1)	502	3	0.6 (0.1–1.7)
Drowsiness	504	105	20.8 (17.4–24.6)	502	149	29.7 (25.7–33.9)	502	100	19.9 (16.5–23.7)
Fever									
Temperature of ≥37.5°C	504	135	26.8 (23.0–30.9)	502	114	22.7 (19.1–26.6)	502	79	15.7 (12.7–19.2)
Temperature of >39°C	504	2	0.4 (0.0–1.4)	502	1	0.2 (0.0–1.1)	502	2	0.4 (0.0–1.4)
Irritability	504	178	35.3 (31.1–39.7)	502	231	46.0 (41.6–50.5)	502	181	36.1 (31.8–40.4)
Loss of appetite	504	75	14.9 (11.9–18.3)	502	110	21.9 (18.4–25.8)	502	82	16.3 (13.2–19.9)
After vaccination visit 4									
Pain									
Measles	163	52	31.9 (24.8–39.6)	161	54	33.5 (26.3–41.4)	159	47	29.6 (22.6–37.3)
Yellow fever	95	2	2.1 (0.3–7.4)	94	7	7.4 (3.0–14.7)	94	2	2.1 (0.3–7.5)
Swelling									
Measles	163	20	12.3 (7.7–18.3)	161	21	13.0 (8.3–19.2)	159	16	10.1 (5.9–15.8)
Yellow fever	95	0	0.0 (0.0–3.8)	94	1	1.1 (0.0–5.8)	94	0	0.0 (0.0–3.8)
Drowsiness	163	30	18.4 (12.8–25.2)	161	50	31.1 (24.0–38.8)	159	34	21.4 (15.3–28.6)
Fever									
Temperature of ≥37.5°C	163	15	9.2 (5.2–14.7)	161	40	24.8 (18.4–32.3)	159	15	9.4 (5.4–15.1)
Temperature of >39°C	163	1	0.6 (0.0–3.4)	161	2	1.2 (0.2–4.4)	159	0	0.0 (0.0–2.3)
Irritability									
All	163	33	20.2 (14.4–27.2)	161	43	26.7 (20.1–34.2)	159	34	21.4 (15.3–28.6)
Grade 3	163	0	0.0 (0.0–2.2)	161	1	0.6 (0.0–3.4)	159	0	0.0 (0.0–2.3)
Loss of appetite	163	33	20.2 (14.4–27.2)	161	47	29.2 (22.3–36.9)	159	33	20.8 (14.7–27.9)

NOTE. Subjects in the RTS,S/AS01$_E$ (0, 1, 2) group received RTS,S/AS01$_E$ at vaccination visits 1, 2, and 3; DTPwHepB/Hib+OPV (diphtheria, tetanus, and whole-cell pertussis conjugate; hepatitis B; *Haemophilus influenzae* type b; and oral polio vaccine) at vaccination visits 1, 2 and 3; and measles and yellow fever vaccines at vaccination visit 4. Subjects in the RTS,S/AS01$_E$ (0, 1, 7) group received RTS,S/AS01$_E$ at vaccination visits 1, 2, and 4; DTPwHepB/Hib+OPV at vaccination visits 1, 2, and 3; and measles and yellow fever vaccines at vaccination visit 4. Subjects in the control group received DTPwHepB/Hib+OPV at vaccination visits 1, 2, and 3 and measles and yellow fever vaccines at vaccination visit 4. CI, confidence interval (exact).

[a] Applicable to solicited local symptoms only.

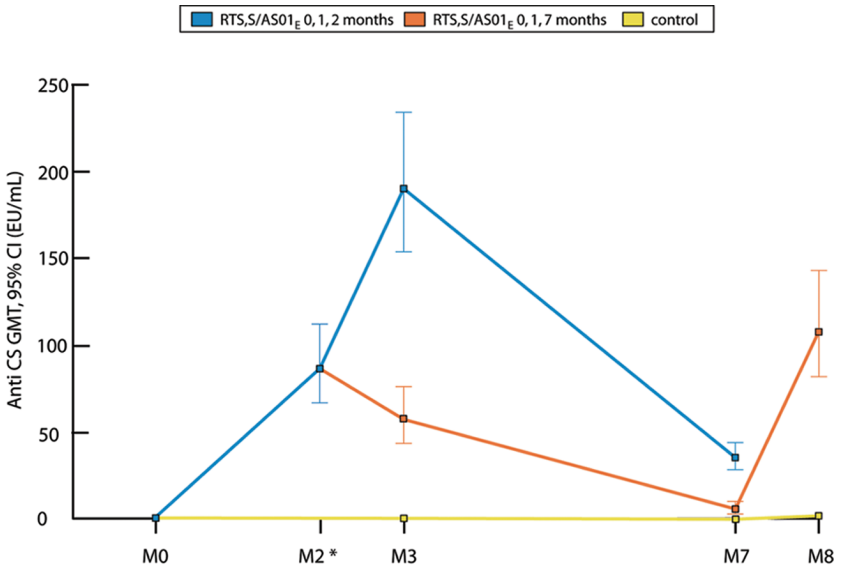

Figure 2. Geometric mean titers (GMTs) at months 0, 2, 3, 7, and 8 of antibodies to *Plasmodium falciparum* circumsporozoite (CS) protein in subjects who received the RTS,S/AS01$_E$ malaria candidate vaccine and control subjects. Subjects in the RTS,S/AS01$_E$ (0, 1, 2) group received RTS,S/AS01$_E$ at study months 0, 1, and 2; subjects in the RTS,S/AS01$_E$ (0, 1, 7) group received RTS,S/AS01$_E$ at study months 0, 1, and 7; and subjects in the control group did not receive RTS,S/AS01$_E$. Error bars show 95% confidence intervals (CIs). *GMTs 1 month after dose 2 (month 2) in the RTS,S/AS01$_E$ (0, 1, 7) group are extrapolated from the RTS,S/AS01$_E$ (0, 1, 2) group.

ter (15.3% in the RTS,S/AS01$_E$ [0, 1, 2] group, 16.5% in the RTS,S/AS01$_E$ [0, 1, 7] group, and 17.0% in the control group). Other unsolicited AEs considered to be causally related to vaccination were as follows: injection site induration in 2 subjects, vomiting in 1 subject, and injection site cellulitis in 1 subject in the RTS,S/AS01$_E$ (0, 1, 2) group; injection site induration in 4 subjects in the RTS,S/AS01$_E$ (0, 1, 7) group; and injection site induration in 4 subjects and cough in 1 subject in the control group. With the exception of the subject who died, no subject withdrew from the study as a result of an AE.

Laboratory safety monitoring. Of the few hematological and biochemical values outside the reference range, 2 values were grade 3 in severity; both occurred in subjects from the control group. One subject had a low hemoglobin concentration (4.1 g/dL) at month 8; no follow-up laboratory safety data were available for this subject, who was reported to have severe sickle cell disease, severe pediatric immune deficiency syndrome, and severe sepsis at the month 7 visit. One subject had an increased level of alanine aminotransferase (444 IU/L) 6 d after dose 1; alanine aminotransferase levels were within the reference range at month 3 and month 8 (25.0 IU/L and 12.6 IU/L, respectively).

Immunogenicity results. Across vaccine groups, 26%–30% of subjects had detectable anti-CS antibodies, at low titers, prior to RTS,S/AS01$_E$ vaccination. At month 3, 99% of subjects in both the RTS,S/AS01$_E$ (0, 1, 2) and RTS,S/AS01$_E$ (0, 1, 7) groups had detectable anti-CS antibodies, compared with a low proportion of anti-CS seropositivity in the control group (11% of subjects). The highest anti-CS GMT of 190.3 EU/mL was observed 1 month after dose 3 (month 3) in the RTS,S/AS01$_E$ (0, 1, 2) group, which compares with a GMT of 107.8 EU/mL 1 month after the third dose (month 8) in the RTS,S/AS01$_E$ (0, 1, 7) group (Figure 2).

Prevaccination anti-HBsAg GMTs were low and similar across vaccine groups (9–13 mIU/mL). At month 3, 100% of subjects in both RTS,S/AS01$_E$ groups and 98% of subjects in the control group had protective levels of anti-HBsAg antibodies (Table 4). The highest GMT (59,814 mIU/mL) was observed at month 8 in the RTS,S/AS01$_E$ (0, 1, 7) group, compared with a GMT of 1356 mIU/mL at month 3 in the RTS,S/AS01$_E$ (0, 1, 2) group and of 338 mIU/mL in the control group.

Noninferiority of anti-HBsAg, anti-diphtheria, anti-tetanus, anti-PRP, anti–polio 1, anti–polio 2, and anti–polio 3 seroprotection rates and anti-BPT antibody titers was demonstrated for the RTS,S/AS01$_E$ (0, 1, 2) and (0, 1, 7) groups, compared with the control group, with the exception of anti–polio 3 in the RTS,S/AS01$_E$ (0, 1, 2) group (Table 4). Noninferiority of anti-measles and anti–yellow fever seroconversion rates was demonstrated for RTS,S/AS01$_E$ coadministered with measles and yellow fever vaccines compared with measles and yellow fever vaccines given alone.

Seroprotective and seropositive antibody titers were similar and high for anti-HBsAg, anti-diphtheria, anti-tetanus, anti-PRP, anti-BPT, and anti–polio (serotypes 1 and 2) (Table 4). Seroprotection rates for anti–polio 3 were slightly lower in participants of the RTS,S/AS01$_E$ (0, 1, 2) group (86.7% of sub-

Table 4. Responses to Antigens from the Expanded Program of Immunization—According to Protocol Cohort for Immunogenicity

Study group, month of assessment, antigen	Rate measured	Noninferiority assessment: No. (%) of subjects[a] RTS,S/AS01$_E$	Noninferiority assessment: No. (%) of subjects[a] Control	Noninferiority assessment: Difference, % (95% CI)[b]	GMT, mIU/mL (95% CI) RTS,S/AS01$_E$	GMT, mIU/mL (95% CI) Control
RTS,S/AS01$_E$ (0, 1, 2), month 3						
HBsAg	SP	130 (100)	126 (97.6)	−2.38 (−6.78 to 0.54)	1356 (1101–1670)	338 (266–429)
Diphtheria	SP	142 (96.5)	142 (100)	3.52 (0.83–7.99)	1.0 (0.9–1.2)	1.4 (1.2–1.7)
Tetanus	SP	142 (100)	142 (100)	0.00 (−2.64 to 2.64)	2.8 (2.3–3.3)	3.7 (3.2–4.3)
Hib PRP	SP	141 (99.3)	142 (98.6)	−0.70 (−4.37 to 2.63)	13.3 (10.6–16.7)	19.0 (15.2–23.6)
Polio 1	SP	136 (94.9)	131 (94.7)	−0.20 (−6.14 to 5.63)	464 (343–627)	500 (365–685)
Polio 2	SP	135 (98.5)	131 (99.2)	0.72 (−2.86 to 4.57)	494 (390–626)	407 (329–503)
Polio 3	SP	135 (86.7)	133 (95.5)	8.82 (2.11–16.16)	124 (92–166)	205 (157–269)
BPT	S+	139 (85.3)[c]	139 (106.5)[c]	0.80 (0.69–0.93)[d]	…	…
RTS,S/AS01$_E$ (0, 1, 7), month 3						
HBsAg	SP	119 (100)	126 (97.6)	−2.38 (−6.78 to 0.80)	651 (541–784)	338 (266–429)
Diphtheria	SP	133 (97.0)	142 (100)	3.01 (0.32–7.49)	1.1 (0.9–1.3)	1.4 (1.2–1.7)
Tetanus	SP	133 (100)	142 (100)	0.00 (−2.64 to 2.82)	2.6 (2.2–3.1)	3.7 (3.2–4.3)
Hib PRP	SP	132 (100)	142 (98.6)	−1.41 (−5.00 to 1.45)	15.6 (12.6–19.4)	19.0 (15.2–23.6)
Polio 1	SP	125 (94.4)	131 (94.7)	0.26 (−5.79 to 6.46)	486 (343–688)	500 (365–685)
Polio 2	SP	124 (100)	131 (99.2)	−0.76 (−4.21 to 2.26)	563 (457–694)	407 (329–503)
Polio 3	SP	125 (92.8)	133 (95.5)	2.69 (−3.30 to 9.19)	149 (112–197)	205 (157–269)
BPT	S+	131 (104.4)[c]	139 (106.5)[c]	0.98 (0.85–1.13)[d]	…	…
RTS,S/AS01$_E$ (0, 1, 7), month 8						
Measles	SC	109 (91.7)	117 (88.9)	−2.85 (−10.95 to 5.23)	1287 (1029–1608)	1267 (1007–1594)
Yellow fever	SC	35 (97.1)	35 (94.3)	−2.86 (−16.30 to 9.67)	203 (134–307)	179 (114–279)

NOTE. Subjects with available postvaccination results were included in the analysis. BPT, *Bordetella pertussis* toxin; CI, confidence interval; GMT, geometric mean titer; HBsAg, hepatitis B surface antigen; Hib, *Haemophilus influenzae* type b; PRP, polyribosyl ribitol phosphate; S+, seropositive rate (percentage of subjects with antibody titers above the seropositive cutoff); SC, seroconversion rate (percentage of initially seronegative subjects who were seropositive after vaccination); SP, seroprotective rate (percentage of subjects with antibody titers above the seroprotective cutoff).

[a] No. (%) of subjects, unless otherwise indicated.
[b] Difference between the percentage of the control group and that of the RTS,S/AS01$_E$ group, unless otherwise indicated.
[c] No. of subjects (GMT).
[d] Ratio of the GMT of the control group to that of the RTS,S/AS01$_E$ group.

jects). Seropositive levels for anti-measles and anti–yellow fever were similar across vaccine groups.

A post hoc analysis of anti-polio responses was conducted to further explore the observed differences in anti–polio 3 responses induced by OPV in coadministration with RTS,S/AS01$_E$ compared with administration of OPV without RTS,S/AS01$_E$. A heterogeneity across vaccine groups of anti–polio 3 titers, but not anti–polio 1 or anti–polio 2 titers (data not shown), was found at screening whereby a higher proportion of seronegative subjects was observed in the RTS,S/AS01$_E$ (0, 1, 2) and RTS,S/AS01$_E$ (0, 1, 7) groups compared with the control group ($P = .015$ and $P = .049$, respectively; Fisher exact test). An analysis of polio 3 antibody responses that took into account titers at screening revealed equivalent vaccine responses across the 3 groups (Table 5).

At month 3, GMTs for anti-diphtheria, anti-tetanus, anti-PRP, anti-BPT, and anti–polio 3 serotypes tended to be lower in the RTS,S/AS01$_E$ coadministration groups than in the control group. This effect was more marked when all 3 doses were given in coadministration (RTS,S/AS01$_E$ [0, 1, 2] group) rather than when 2 doses were coadministered (RTS,S/AS01$_E$ [0, 1, 7] group).

DISCUSSION

This is the first study of the RTS,S/AS01$_E$ malaria candidate vaccine integrated into the EPI schedule, with vaccinations starting at 6 weeks of age. Overall, the study showed a favorable safety assessment of RTS,S/AS01$_E$ incorporation into the EPI schedule, which is in line with previous results in which the closely related RTS,S/AS02$_D$ vaccine was coadministered with EPI vaccines with a tetravalent DTPw/Hib vaccine in Tanzania [9]. Mild or moderate fever was reported more frequently in RTS,S/AS01$_E$ coadministered groups than in control groups with EPI vaccines given alone, but grade 3 fever was rare in any of the study groups. The occurrence of SAEs during the whole reporting period and that of unsolicited AEs within 30 d after vaccination were reported in a similar proportion of

Table 5. Seropositivity rates, Geometric Mean Titers (GMTs), and Vaccine Response for Anti–Polio 3 Antibodies in the According to Protocol Cohort for Immunogenicity

Study group, timing or seropositivitystatus at screening	No. of subjects	% (95% CI)	GMT (95% CI)	Vaccine response, % (95% CI)
	Anti–polio 3 antibody responses over time			
RTS,S/AS01$_E$ (0, 1, 2)				
Screening	128	47.7 (38.8–56.7)	14.2 (10.7–18.9)	…
Month 3	112	88.4 (81.0–93.7)	130.4 (95.9–177.3)	75.0 (65.9–82.7)
RTS,S/AS01$_E$ (0, 1, 7)				
Screening	117	50.4 (41.0–59.8)	16.1 (12.0–21.8)	…
Month 3	100	93.0 (86.1–97.1)	149.1 (110.3–201.5)	78.0 (68.6–85.7)
Control				
Screening	118	63.6 (54.2–72.2)	25.4 (18.4–35.1)	…
Month 3	97	94.8 (88.4–98.3)	199.2 (144.6–274.5)	73.2 (63.2–81.7)
	Anti–polio 3 antibody responses at month 3 according to seropositivity status at screening			
RTS,S/AS01$_E$ (0, 1, 2)				
Seronegative	60	85.0 (73.4–92.9)	94.2 (61.2–145.1)	85.0 (73.4–92.9)
Seropositive	52	92.3 (81.5–97.9)	189.8 (123.7–291.0)	63.5 (49.0–76.4)
RTS,S/AS01$_E$ (0, 1, 7)				
Seronegative	52	92.3 (81.5–97.9)	120.5 (80.0–181.5)	92.3 (81.5–97.9)
Seropositive	48	93.8 (82.8–98.7)	187.7 (119.5–294.7)	62.5 (47.4–76.0)
Control				
Seronegative	36	94.4 (81.3–99.3)	142.2 (84.3–239.8)	94.4 (81.3–99.3)
Seropositive	61	95.1 (86.3–99.0)	243.1 (161.6–365.8)	60.7 (47.3–72.9)

NOTE. Subjects with both prevaccination and postvaccination results were included. Seronegative subjects had an antibody titer of <8 times the median effective dose (ED_{50}) prior to vaccination. Seropositive subjects had an antibody titer of ⩾8 ED_{50} prior to vaccination. For initially seronegative subjects, vaccine response was defined as an antibody titer at month 3 of ⩾8 ED_{50}. For initially seropositive subjects, vaccine response was defined as an antibody titer at month 3 of ⩾2-fold the prevaccination antibody titer. Similar results were obtained when a 4-fold increase criterion was used (data not shown). CI, confidence interval; GMT, geometric mean antibody titer.

subjects in each study group; unsolicited AEs related to vaccination were predominantly due to reports of induration at 1 study center that were balanced across groups.

This study also assessed the antibody responses to coadministered EPI antigens. As in a previously reported trial in Tanzania [9], GMTs to diphtheria, tetanus, BPT, Hib, and polio 3 antigens were slightly lower in the RTS,S/AS01$_E$ coadministration groups than in the control group, more so when all 3 DTPwHepB/Hib+OPV vaccine doses were coadministered (RTS,S/AS01$_E$ [0, 1, 2] group) than when 2 doses were coadministered (RTS,S/AS01$_E$ [0, 1, 7] group). There was no interference with polio 1, polio 2, measles, and yellow fever responses upon coadministration. Overall, the seropositivity and seroprotection rates were high and in accordance with the expected rates of EPI antigen responses in resource-limited countries [20–24], and the predefined noninferiority criteria were met for the DTPwHepB/Hib+OPV, measles, and yellow fever antigens, except for polio 3 antigen in the RTS,S/AS01$_E$ (0, 1, 2) group.

An apparent interaction between an oral live attenuated vaccine and a recombinant injected vaccine was unexpected, and this finding may have been due to chance in the context of the multiple comparisons that were made. A post hoc analysis of the screening samples supports the view that the differences in the response to anti–polio 3 between groups may be related to a heterogeneity across groups in anti–polio 3 antibody levels at screening: there was a higher proportion of polio 3 seronegative subjects and lower polio 3 GMTs in both RTS,S/AS01$_E$ groups at screening, in comparison with the control group. When polio type 3 immune responses were analyzed taking into account antibody levels at screening, as is frequently done when assessing polio immunization responses in infants [25, 26], similar vaccine response rates were observed in all 3 groups. Further evaluation of OPV responses are planned as part of the ongoing phase 3 RTS,S/AS01$_E$ clinical trial.

In terms of anti-CS responses, the results of this study confirm the induction of high anti-CS antibody responses by RTS,S/AS01$_E$ vaccination; levels were well above the minimal responses induced by natural parasite exposure. The peak response following the third dose in both vaccination schedules in the study was higher than the peak following 2 doses, which supports previous results showing the superiority of a 3-dose schedule compared with a 2-dose schedule [12]. Also in line with results of a previous study in children aged 5–17 months

in Ghana [11], this study confirmed that a higher peak anti-CS response is induced by a schedule of vaccination at 0, 1, and 2 months compared with a schedule at 0, 1, and 7 months.

The RTS,S antigen is a recombinant construct that also expresses HBsAg. This study shows that RTS,S/AS01$_E$ can be incorporated into the EPI with another HepB-containing combination vaccine without generating safety concerns. Higher anti-HBsAg GMTs were found in the RTS,S/AS01$_E$ groups compared with those in the control group, which might be associated with the induction of longer lasting protection. Unlike the CS response, the HBsAg response was highest when the third RTS,S/AS01$_E$ dose was delayed—in the schedule of vaccination at 0, 1, and 7 months compared with that at 0, 1, and 2 months, as was previously found in a RTS,S schedule study among slightly older children [11]. This is in line with a well-described characteristic of hepatitis B vaccination responses: the immune response to a delayed last immunization is higher than when it follows the previous doses closely [27, 28].

In summary, this trial has shown a favorable safety and immunogenicity evaluation of the RTS,S/AS01$_E$ malaria candidate vaccine introduced into the EPI on a schedule of vaccination at 0, 1, and 2 months or on a schedule at 0, 1, and 7 months. Because a schedule at 0, 1, and 2 months can be readily implemented in the EPI and may be associated with higher coverage compared with a schedule at 0, 1, and 7 months, the schedule at 0, 1, and 2 months has been selected for further assessment in the ongoing RTS,S/AS01$_E$ phase 3 efficacy study.

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