Safety and efficacy of the RTS,S/AS01$_E$ candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial

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Summary

Background The RTS,S/AS01$_E$ candidate malaria vaccine is being developed for immunisation of infants in Africa through the expanded programme on immunisation (EPI). 8 month follow-up data have been reported for safety and immunogenicity of RTS,S/AS01$_E$ when integrated into the EPI. We report extended follow-up to 19 months, including efficacy results.

Methods We did a randomised, open-label, phase 2 trial of safety and efficacy of the RTS,S/AS01$_E$ candidate malaria vaccine given with EPI vaccines between April 30, 2007, and Oct 7, 2009, in Ghana, Tanzania, and Gabon. Eligible children were 6–10 weeks of age at first vaccination, without serious acute or chronic illness. All children received the EPI diphtheria, tetanus, pertussis (inactivated whole-cell), and hepatitis-B vaccines, *Haemophilus influenzae* type b vaccine, and oral polio vaccine at study months 0, 1, and 2, and measles vaccine and yellow fever vaccines at study month 7. Participants were randomly assigned (1:1:1) to receive three doses of RTS,S/AS01$_E$ at 6, 10, and 14 weeks (0, 1, 2 month schedule) or at 6 weeks, 10 weeks, and 9 months (0, 2, 7 month schedule) or placebo. Randomisation was according to a predefined block list with a computer-generated randomisation code. Detection of serious adverse events and malaria was by passive case detection. Antibodies against *Plasmodium falciparum* circumsporozoite protein and HBsAg were monitored for 19 months. This study is registered with ClinicalTrials.gov, number NCT00436007.

Findings 511 children were enrolled. Serious adverse events occurred in 57 participants in the RTS,S/AS01$_E$ 0, 1, 2 month group (34%, 95% CI 27–41), 47 in the 0, 1, 7 month group (28%, 21–35), and 49 (29%, 22–36) in the control group; none were judged to be related to study vaccination. At month 19, anticircumsporozoite immune responses were significantly higher in the RTS,S/AS01$_E$ groups than in the control group. Vaccine efficacy for the 0, 1, 2 month schedule (2 weeks after dose three to month 19, site-adjusted according-to-protocol analysis) was 53% (95% CI 26–70; p=0·0012) against first malaria episodes and 59% (36–74; p=0·0001) against all malaria episodes. For the entire study period, (total vaccinated cohort) vaccine efficacy against all malaria episodes was higher with the 0, 1, 2 month schedule (57%, 95% CI 33–73; p=0·0002) than with the 0, 1, 7 month schedule (32% CI 16–45; p=0·0003). 1 year after dose three, vaccine efficacy against first malaria episodes was similar for both schedules (0, 1, 2 month group, 61·6% [95% CI 35·6–77·1], p<0·001; 0, 1, 7 month group, 63·8% [40·4–78·0], p<0·001, according-to-protocol cohort).

Interpretation Vaccine efficacy was consistent with the target put forward by the WHO-sponsored malaria vaccine technology roadmap for a first-generation malaria vaccine. The 0, 1, 2 month vaccine schedule has been selected for phase 3 candidate vaccine assessment.

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See Comment page 722

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Introduction

The devastating morbidity and mortality that results from malaria in sub-Saharan Africa, especially in children less than 5 years of age, is well documented.[1] The development of malaria vaccines has been identified by national and international health authorities as a key component of a sustainable malaria control programme, which will have large benefits for health and the economy.[2–4]

The RTS,S/AS candidate malaria vaccine is being developed for immunisation of infants and children living in malaria-endemic areas in sub-Saharan Africa as part of the expanded programme on immunisation (EPI). The vaccine has been assessed with two different proprietary adjuvant systems, AS02 and AS01, both having shown a promising safety profile in children[5–9] and infants.[10–12] The RTS,S antigen includes a carboxy-terminal segment of

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the *Plasmodium falciparum* circumsporozoite protein fused to the HBsAg. Simultaneously expressed in yeast cells together with free HBsAg, these antigens assemble into particulate structures. The $AS01_E$ adjuvant system contains the immunostimulants MPL and QS21 and liposomes.

A phase 2 trial[12] in Gabon, Ghana, and Tanzania in infants aged 6–10 weeks at first vaccination was done to assess safety, immunogenicity, and efficacy of two $RTS,S/AS01_E$ schedules (0, 1, 2 month and 0, 1, 7 month schedules) for potential integration into the EPI. Malaria transmission is intense and perennial in these regions,[9,13–15] although the burden of malaria has recently decreased in Lambaréné, Gabon.[16,17] The primary analysis of safety and immunogenicity of the vaccine up to study-month 8 h as been reported.[12] The incidence of serious adverse events was balanced across groups and none were judged to be related to vaccination. Anticircumsporozoite and anti-HBsAg antibody responses were high in $RTS,S/AS01_E$ recipients. At administration of $RTS,S/AS01_E$ with EPI antigens, seroprotection and seroconversion rates were high, and predefined non-inferiority criteria were met for all EPI antigens with the exception of polio 3, although when antibody concentrations measured at screening were taken into account, the rates of response to polio 3 were comparable between groups.[12] We assessed the safety, immunogenicity, and efficacy of $RTS,S/AS01_E$ over 19 months, follow-up.

Methods

Study design and participants

Full study design and enrolment details have been published (webappendix 1).[12] We did a randomised, open-label, phase 2 trial. Participants received EPI vaccines alone (control group) or together with $RTS,S/AS01_E$ in two different dosing schedules (table 1).

See Online for webappendix 1

Eligible children were 6–10 weeks of age at first vaccination, without serious acute or chronic illness established by clinical or physical examination, medical history records, or laboratory screening tests (haematological analysis, renal function, and hepatic function). All participants must have received neonatal oral polio vaccine and BCG through national immunisation programmes. Long lasting insecticide-impregnated bednets were distributed at screening.

Written informed consent was obtained from each child's parent(s) before study procedures were initiated. Illiterate parents indicated consent with a thumbprint, and a signature was obtained from a literate witness.

The trial was done in accordance with the Helsinki Declaration of 1964 (revised in 1996)[18] and according to Good Clinical Practice guidelines.[19] Approval was obtained from the local and national ethics committees relevant to each site, the London School of Hygiene and Tropical Medicine Ethics Committee, UK, the Swiss Tropical Institute Committee, Switzerland, and The Western Institutional Review Board, USA. The design, conduct, and results of the trial were overseen by a formally constituted independent data monitoring committee. The study was done under US Food and Drug Administration and national regulatory oversight as per existing regulations. The Ghana Food and Drugs Board, the National Institute for Medical Research of Tanzania, and the Ministry of Health in Gabon reviewed and approved the study before it started.

All children received diphtheria, tetanus, pertussis (inactivated whole-cell), hepatitis B vaccine (Tritanrix, GlaxoSmithKline Biologicals, Rixensart, Belgium), *Haemophilus influenzae* type b vaccine (Hiberix, GlaxoSmithKline Biologicals, Rixensart, Belgium), and oral polio vaccine (Polio Sabin, GlaxoSmithKline Biologicals, Rixensart, Belgium) at study months 0, 1, and 2, and measles vaccine (Rouvax, Sanofi Pasteur, Lyon, France; some participants from Gabon received measles vaccine sourced from the EPI [Serum Institute of India]), and yellow fever vaccine (Stamaril, Sanofi Pasteur, Lyon, France; excluding Tanzania where yellow fever vaccination was not included in the national EPI) at study month 7.

Randomisation and masking

Participants were randomly assigned (1:1:1) to one of the two $RTS,S/AS01_E$ vaccine groups or control according to a computer generated predefined block randomisation list (table 1). The study was an open design; the investigators,

	Month 0*	Month 1†	Month 2‡	Month 3	Month 7§	Month 8	Month 19
$RTS,S/AS$ 0, 1, 2 months (n=170)	DTPwHepB/Hib, OPV, and $RST,S/AS01_E$	DTPwHepB/Hib, OPV, and $RST,S/AS01_E$	DTPwHepB/Hib, OPV, and $RST,S/AS01_E$	..	Measles and yellow fever vaccines	..	..
Blood sampling	Yes	No	Yes	Yes	Yes	No	Yes
$RTS,S/AS$ 0, 1, 7 months (n=170)	DTPwHepB/Hib, OPV, and $RST,S/AS01_E$	DTPwHepB/Hib, OPV, and $RST,S/AS01_E$	DTPwHepB/Hib and OPV	..	Measles and yellow fever vaccines¶ and $RST,S/AS01_E$	..	..
Blood sampling	Yes	No	No	Yes	Yes	Yes	Yes
Control group (n=171)	DTPwHepB/Hib + OPV	DTPwHepB/Hib + OPV	DTPwHepB/Hib + OPV	..	Measles and yellow fever vaccines	..	..
Blood sampling	Yes	No	No	Yes	Yes	Yes	Yes

DTPwHepB/Hib=diphtheria, tetanus, pertussis (inactivated whole-cell) vaccine, hepatitis B vaccine, and *Haemophilus influenzae* type b vaccine. OPV=oral polio vaccine. *Age at vaccination was 6 weeks. †Age at vaccination was 10 weeks. ‡Age at vaccination was 14 weeks. §Age at vaccination was 9 months. ¶Except participants from Tanzania.

***Table 1*: Study groups and sampling timepoints**

study personnel and participants were not blinded to the allocated study vaccines.

Procedures

Parents were instructed to go to study facilities or local health centres when their child was unwell. Study doctors were available 24 h a day to attend to sick children and record all visits and occurrences of serious adverse events, defined per protocol as any untoward medical occurrence that was fatal, life-threatening, required hospitalisation, led to disability or incapacity, or was judged by investigators as being medically important enough to be reported as serious. After 3 months of study, reporting of these events was improved by means of monthly home-visits by field workers. In the case of a death, supplementary information was obtained by verbal autopsy.[20]

Severe malaria was recorded as part of safety surveillance and was defined as *P falciparum* asexual parasitaemia and at least one of these symptoms: haemoglobin <50 g/L, coma score of two or more, multiple seizures, prostration, hypoglycaemia, acidosis or circulatory collapse, and no other more probable cause of illness. Cases of *P falciparum* malaria (including cases of severe malaria) reported as serious adverse advents were coded in MedDRA as "*P falciparum* infection".[21]

Blood specimens for safety assessment (complete blood count, alanine transaminase, creatinine) were collected at study-month 19. For assessment of immunogenicity, we measured antibody titres for anticircumsporozoite protein and anti-HBsAg at study-month 19. Serum antibodies to the NANP repeat region of circumsporozoite proteins (B-cell epitope) were measured by a standard, validated ELISA with plates adsorbed with the recombinant antigen R32LR, which contains the sequence $[NVDP(NANP)_{15}]_2LR$. We calculated antibody titres with a reference standard curve with a four-parameter logistic-fitting algorithm and expressed in ELISA units (EU) per mL, with a cutoff for seropositivity set at 0·5 EU/mL. We measured concentrations of anti-HBsAg with an in-house ELISA.[22]

To further characterise the anti-hepatitis B immune response to RTS,S/AS01$_E$ given with another EPI hepatitis-B vaccine, we assessed antibody titres for anti-RF1 1 month after the third dose of vaccine 3 months into the study in the RTS,S/AS01$_E$ 0, 1, 2 month group and the control group. Antibody responses to the RF1 epitope on the HBsAg are indicative of the virus-neutralising capacity of the humoral immune response, as shown by use of a monoclonal antibody against the RF1 epitope to protect against experimental hepatitis-B infection in animal models.[23] We measured RF1-like antibody concentrations using an in-house ELISA-based competition assay with adsorbed HBsAg. Dilutions of the test samples and the reference serum were mixed with a fixed amount of RF1 monoclonal antibodies, which was identified by colorimetric reaction. We quantified the amount of antibodies competing with RF1 monoclonal antibodies for binding to the coated HBsAg by comparison with a reference serum using a four-parameters equation (Softmax Pro Software), with an assay cutoff of 33 EU/mL.

For assessment of vaccine efficacy, malaria episodes were detected by passive case detection: parents or guardians were asked to go to a study health facility when their child was unwell. If the child had a history of fever or a temperature recording of 37·5°C or more, a blood sample was taken and a blood slide examined. Efficacy assessment was a protocol amendment added as an exploratory objective, but this did not need any modification or addition of already implemented procedures for participants. The information required was history of fever in the previous 24 h, temperature measurement, and detection of malaria parasitaemia (in case of fever), all of which were part of routine clinical assessment of sick children presenting at the study health centres. All efficacy data recorded before local approval of the protocol amendment were extracted from clinical records retrospectively, and data recorded after local approval were recorded prospectively. Vaccine efficacy against severe malaria was not a study endpoint; severe malaria episodes were reported as part of safety surveillance.

Statistical analysis

A statistical analysis plan was agreed by the independent data monitoring committee, study sponsor, and investigators before database closure (webappendix 2). Statistical analyses were done with SAS version 9.1. The sample size was driven by the immunogenicity non-inferiority endpoints for co-administered EPI vaccines.[12]

See Online for webappendix 2

Safety analysis was done on the total vaccinated cohort (ie, all vaccinated children for whom data were available). The proportion of those with a serious adverse event, as classified by the MedDRA preferred-term level, reported from 0–19 months was tabulated with exact 95% CIs.

The frequency distributions of biochemical (alanine transaminase and creatinine) and haematological (haemoglobin, white blood cells, platelets) laboratory values outside of predefined reference ranges were analysed by predefined severity grades. The immunogenicity analysis was done on the according-to-protocol cohort (ie, children meeting all eligibility criteria, complying with protocol-defined procedures, with no elimination criteria and for whom immunogenicity data were available). Percentages of children seropositive for anticircumsporozoite (≥0·5 EU/mL), seroprotective for anti-HBs (≥10 mIU/mL), and seropositive for anti-RF1 (≥33 EU/mL) were determined. Antibody titres were summarised by geometric mean titres (GMT) with 95% CIs.

The study had 80% power to show a vaccine efficacy of 40% if the attack rate in the control group was 0·5 episode per child-year at risk. Analyses of vaccine efficacy were done on the according-to-protocol cohort (ie, participants who received all doses of study vaccines and contributed

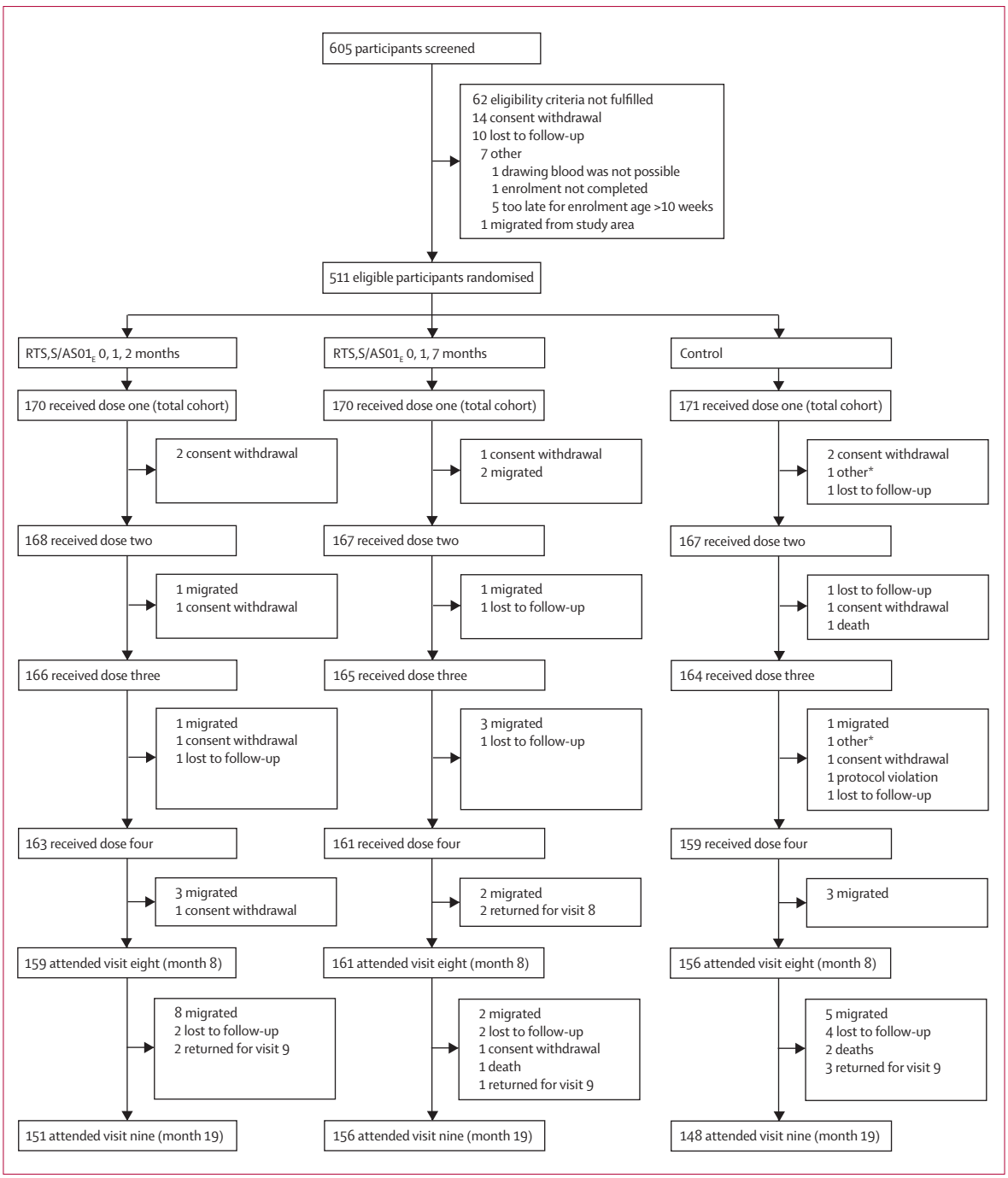

Figure 1: **Trial profile**
*These participants were withdrawn by the investigators because they were unlikely to comply with study procedures.

to the efficacy follow-up). Analyses of vaccine efficacy during the whole study period were done on the total vaccinated cohort.

We assessed vaccine efficacy against cases of symptomatic *P falciparum* malaria meeting primary or secondary case definitions. The primary case definition was *P falciparum* asexual parasitaemia of more than 500 parasites per μL with fever (axillary temperature ≥37·5°C) at presentation of a sick child at a health-care facility. The secondary case definition was *P falciparum* asexual parasitaemia with any parasites per μL blood sample with fever (axillary temperature ≥37·5°C) at presentation or history of fever within 24 h of presentation of a sick child at a health-care facility.

Efficacy estimates, both crude and adjusted (for study site), were obtained for the first or only and for all episodes of *P falciparum* malaria. For analyses of first or only episodes, vaccine efficacy was assessed using Cox regression models, defined as 1 minus R, where R was the hazard ratio of the RTS,S/AS01$_E$ group versus the control group (with 95% CI). For analysis of all episodes, vaccine efficacy was defined as 1 minus R, where R was the rate ratio of clinical episodes of the RTS,S/AS01$_E$ group versus the control group (with 95% CI). Vaccine efficacy was assessed using Poisson regression models with random effects including the time at risk as an offset variable.

To assess persistence of efficacy to month 19, the assumption of proportionality of hazards was assessed with Schoenfeld residuals (ie, correlation between scaled Schoenfeld residual and rank of time).

Anticircumsporozoite immune responses in RTS,S recipients were catagorised into children with at least one episode of malaria and those with none. Immune responses were compared with Wilcoxon rank-sum tests. Hazard rates per ten-times increase in anticircumsporozoite responses and hazard rates of high versus low tertile of anticircumsporozoite responses in RTS,S/AS01$_E$ recipients were expressed as percentage reduction in risk of malaria episodes (1 minus HR); p values were calculated from the Cox model.

Role of the funding source

GlaxoSmithKline (GSK) Biologicals was responsible for the study design with input from the Malaria Vaccine Initiative from the Program for Appropriate Technology in Health and investigators. Data collection and entry was done at the study sites by study personnel. Data analysis was done at GSK according to the agreed predefined analysis plan. GSK Biologicals and the Malaria Vaccine Initiative contributed to data interpretation, reporting, and publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	RTS,S/AS01$_E$ (0, 1, 2 months; n=170)	RTS,S/AS01$_E$ (0, 1, 7 months; n=170)	Control group (n=171)
Any*	57 (33·5% [26·5–41·2])	47 (27·6% [21·1–35·0])	49 (28·7% [22·0–36·1])
Anaemia	11 (6·5% [3·3–11·3])	13 (7·6% [4·1–12·7])	16 (9·4% [5·4–14·7])
Bronchitis	4 (2·4% [0·6–5·9])	3 (1·8% [0·4–5·1])	1 (0·6% [0·0–3·2])
Gastroenteritis	23 (13·5% [8·8–19·6])	16 (9·4% [5·5–14·8])	16 (9·4% [5·4–14·7])
Impetigo	5 (2·9% [1·0–6·7])	3 (1·8% [0·4–5·1])	6 (3·5% [1·3–7·5])
Otitis media	4 (2·4% [0·6–5·9])	0 (0·0% [0·0–2·1])	2 (1·2% [0·1–4·2])
Plasmodium falciparum infection	11 (6·5% [3·3–11·3])	14 (8·2% [4·6–13·4])	23 (13·5% [8·7–19·5])
Pneumonia	15 (8·8% [5·0–14·1])	14 (8·2% [4·6–13·4])	15 (8·8% [5·0–14·1])
Sepsis	3 (1·8% [0·4–5·1])	2 (1·2% [0·1–4·2])	4 (2·3% [0·6–5·9])
Upper-respiratory-tract infection	12 (7·1% [3·7–12·0])	8 (4·7% [2·1–9·1])	9 (5·3% [2·4–9·8])
Urinary-tract infection	6 (3·5% [1·3–7·5])	2 (1·2% [0·1–4·2])	1 (0·6% [0·0–3·2])
Febrile convulsion	1 (0·6% [0·0–3·2])	6 (3·5% [1·3–7·5])	0 (0·0% [0·0–2·1])

Data are number (% [95% CI]) of participants given at least one dose of vaccine with at least one serious adverse event.
*At least one symptom experienced (regardless of the MedDRA preferred term).

Table 2: **Serious adverse events occurring in at least 2% of subjects in any vaccine group (months 0–19, total vaccinated cohort)**

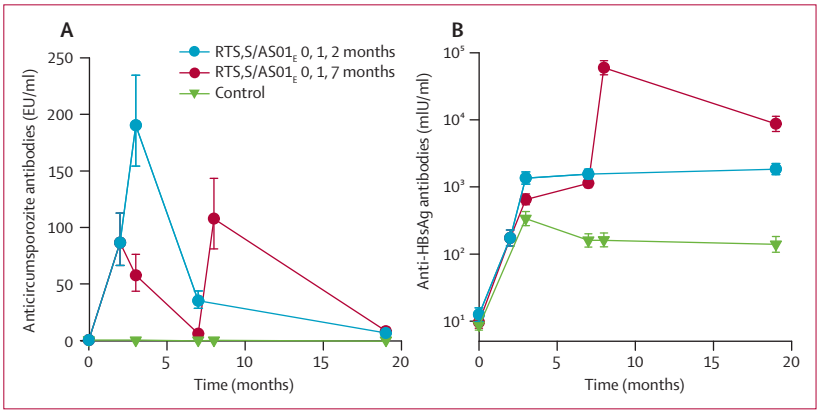

Figure 2: **Anticircumsporozoites and anti-HBs antibody titres in recipients of RTS,S/AS01$_E$ (0, 1, 2 month and 0, 1, 7 month groups) and the control group**
EU=ELISA units.

Results

Participants were enrolled between April 30, 2007, and Oct 7, 2009, at Kintampo Health Research Centre, Kintampo, Ghana, Ifakara Health Research and Development Centre, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania, and the Albert Schweitzer Hospital, Medical Research Unit, Lambaréné, Gabon. 511 infants (220 Gabon, 81 Ghana, 210 Tanzania) were enrolled and received at least one vaccination (figure 1). The month-19 visit was completed by 455 children (89%); the main reason for withdrawal across groups was migration from the study area.

At month 0, the demographic profile of children across groups was balanced in terms of age and sex; mean age was 7·0 weeks (SD 1·0), and 51% of participants were boys.[12] Use of bednets was not monitored during the study. In the communities, no insecticide spraying campaign occurred and intermittent preventive malaria treatment was not implemented at the time of study.

From study months 0–19, the proportion of children with at least one serious adverse event was similar in each group (table 2). Although hospital admission was not a study endpoint, these figures reflect hospital admissions because apart from one fatality at home, hospitalisation was the criteria that triggered serious adverse event reporting for all serious adverse events, and all hospitalisations were reported as serious adverse events. No serious adverse event was judged to be related to vaccination and no individual event occurred with a higher incidence that was of clinical concern in either RTS,S/AS01$_E$ group compared with the control group.

11 cases of *P falciparum* infection were reported as serious adverse events in the RTS,S/AS01$_E$ 0, 1, 2 month group, 14 in RTS,S/AS01$_E$ 0, 1, 7 month group, and 23 in

	RTS,S/AS01$_E$			Control			Vaccine efficacy adjusted*		Vaccine efficacy unadjusted	
	n	Events	Rate	n	Events	Rate	% (95% CI)	p value	% (95% CI)	p value
Months 2·5-19 (0, 1 , 2 month group; according-to-protocol cohort)										
First or only malaria episode										
Primary case definition	159	30	0·15	156	52	0·30	52·5 (25·5 to 69·7)	0·001	48·5 (19·3 to 67·4)	0·004
Secondary case definition	159	38	0·202	156	53	0·32	41·5 (11·3 to 61·5)	0·012	36·3 (3·3 to 58·0)	0·034
All malaria episodes										
Primary case definition	159	43	0·20	156	102	0·49	59·1 (35·8 to 73·9)	0·0001	60·6 (33·3 to 76·7)	0·001
Secondary case definition	159	73	0·35	156	146	0·71	53·1 (24·4 to 70·9)	0·002	53·8 (19·0 to 73·6)	0·007
1 year after dose three (according-to-protocol cohort)										
0,1, 2 month group (months 2·5–14)										
First or only†	159	21	0·15	156	46	0·36	61·6 (35·6 to 77·1)	0·0003	58·7 (30·7 to 75·3)	0·0008
0,1, 7 month group (months 7·5–19)										
First or only†	154	23	0·17	153	48	0·43	63·8 (40·4 to 78·0)	<0·0001	58·7 (32·0 to 74·9)	0·0005
Early vaccine efficacy (according-to-protocol cohort)										
0,1, 2 month group (months 2·5–8)										
First or only†	159	9	0·12	155	23	0·33	66·7 (27·2 to 84·8)	0·006	62·0 (17·5 to 82·5)	0·014
0, 1, 7 month group (months 1·5–7)										
First or only†	154	15	0·21	159	17	0·23	15·2 (–70·2 to 57·7)	0·643	12·7 (–74·9 to 56·4)	0·702
Total study duration (months 0-19; total vaccinated cohort)										
0, 1, 2 month group (all malaria episodes)										
Primary case definition	170	46	0·18	171	106	0·42	57·2 (33·1 to 72·7)	0·0002	58·6 (30·2 to 75·4)	0·001
Secondary case definition	170	77	0·30	171	154	0·62	51·3 (22·5 to 69·4)	0·0025	52·2 (17·4 to 72·3)	0·008
0, 1, 7 month group (all malaria episodes)										
Primary case definition	170	54	0·21	171	106	0·42	32·0 (16·4 to 44·7)	0·0003	30·6 (11·3 to 45·6)	0·004
Secondary case definition	170	85	0·33	171	154	0·62	30·2 (13·6 to 43·7)	0·001	26·6 (4·9 to 43·4	0·020

*Adjusted estimates for site. †Primary case definition.

***Table 3*: Vaccine efficacy against *P falciparum* malaria**

the control group (table 2). One case of severe malaria was reported in the RTS,S/AS01$_E$ 0, 1, 2 month group, three in the RTS,S/AS01$_E$ 0, 1, 7 month group, and five in the control group. The proportion of children with at least one serious adverse event remained similar in each group after exclusion of malaria: 32·4% (95% CI 25·4–39·9) in the RTS,S/AS01$_E$ 0, 1, 2 month group, 26·5% (20–33·8) in the RTS,S/AS01$_E$ 0, 1, 7 month group, and 26·9% (20·4–34·2) in the control group.

Anaemia reported as a serious adverse event occurred with a similar frequency across groups. Seven children had at least one febrile convulsion: one in the RTS,S/AS01E 0, 1, 2 month group and six in the RTS,S/AS01E 0, 1, 7 month group. None occurred within a week post vaccination. Four children died: three in the control group (one died of pneumonia and malaria; one of HIV/AIDS, severe malnutrition, pneumonia, and sepsis; and one of suspected leukaemia, suspected HIV/AIDS, anaemia, septicaemia, and malaria) and one in the RTS,S/AS01$_E$ 0, 1, 7 month group (severe gastroenteritis).

Of the few haematological and biochemical values outside the normal range, two were grade 3 in severity at month 19, both in the RTS,S/AS01$_E$ 0, 1, 7 month group: one child had a low platelet count (<25×10^3/μL) and one had severe aneamia (<5·0 g/dL). The mean haemoglobin concentration at month 19 was similar across groups (data not shown).

26–30% of participants across vaccine groups were seropositive (detectable concentrations) for anticircumsporozoite antibodies, at very low titres, before vaccination. At month 19, 118 (94% [95% CI 88–97]) children in the RTS,S/AS01$_E$ 0, 1, 2 month group and 104 (85% [77–90]) in the RTS,S/AS01$_E$ 0, 1, 7 month group were seropositive for anticircumsporozoite antibodies, compared with six (5% [2–11]) in the control group. The highest anticircumsporozoite antibody GMT was reported at month 3 in the RTS,S/AS01$_E$ 0, 1, 2 month group. At month 19, anticircumsporozoite antibody GMTs in both RTS,S/AS01$_E$ groups remained higher than those in the control group (figure 2).

25–38% of children across vaccine groups had seroprotective anti-HBs antibody concentrations before vaccination; antibody GMTs were low and similar across groups (9–13 mIU/mL). At month 19, 249 (100% [95% CI 97–100]) recipients of RTS,S/AS01$_E$ and 116 (97% [92–99]) who received EPI vaccines only were seroprotected against hepatitis-B-virus infection.

The highest anti-HBs antibody concentrations were measured at month 8 in the RTS,S/AS01$_E$ 0, 1, 7 month group. At month 19, the highest anti-HBs GMT,

8748 mIU/mL, was measured in the RTS,S/AS01$_E$ 0, 1, 7 month group, which compared with 1845 mIU/mL in the RTS,S/AS01$_E$ 0, 1, 2 month group and 140 mIU/mL in the control group (figure 2).

At month 3, 48 (96%) of 50 children were seropositive for anti-RF1 antibodies in the RTS,S/AS01$_E$ 0, 1, 2 month group (95% CI 86·3–99·5) and 33 (66%) of 50 were seropostive in the control group (51·2–78·8); anti-RF1 antibody GMT was higher in those in the RTS,S/AS01$_E$ 0, 1, 2 month group (230·8 [95% CI 165–323]) than in the control group (42·4 [33·7–53·4]).

The risk of malaria across study centres differed. In the control group, six episodes of malaria (primary case definition; all events, months 0–19) occurred in Gabon, 46 in Ghana, and 54 in Tanzania. Incidence of malaria was 0·05 episodes per person-years at risk in Gabon, 1·3 in Ghana, and 0·52 in Tanzania. In total, 95 (77%) of 123 first malaria episodes were recorded prospectively and 28 (23%) of 123 retrospectively.

Table 3 shows the various vaccine efficacy analyses. Figure 3 shows the cumulative proportion of children with at least one episode of *P falciparum* malaria (primary case definition) in the three study groups, during the whole study duration (total vaccinated cohort). Site-adjusted vaccine efficacy against malaria (primary case definition) in the RTS,S/AS01E 0, 1, 2 month group from months 2·5 to 19 was 52·5% (p=0·001) for first or only malaria episodes and 59·1% (p=0·0001) for all malaria episodes (according-to-protocol cohort). When assessed up to 1 year after RTS,S/AS01E dose three, vaccine efficacy in the 0, 1, 2 month group (months 2·5 to 14) and in the 0, 1, 7 month group (months 7·5 to 19) were equivalent. Throughout the entire study period (months 0 to 19, total vaccinated cohort), the point estimate of vaccine efficacy in the 0, 1, 2 month group was higher than in the 0, 1, 7 month group. The point estimate of vaccine efficacy 6 months after the third dose from months 2·5 to 8 in the 0, 1, 2 month group was higher than after two doses from months 1·5 to 7 before administration of the third dose in the 0, 1, 7 month group. Vaccine effect did not wane from months 2·5 to 19 in the 0, 1, 2 month group (p=0·14) or months 7·5 to 19 in the 0, 1, 7 month group (p=0·26).

In the RTS,S/AS01$_E$ 0, 1, 2 month group, anticircumsporozoite antibody GMTs were higher in children who did not have an episode of *P falciparum* malaria than in those who did, at all timepoints assessed from months 2·5–19 (day 60 p<0·0001; month 3 p=0·0005; month 7 p<0·0001; month 19 p<0·0001). A ten-times increase in anticircumsporozoite antibody titres at month 3 was associated with a 13·5% reduction in the risk of a new episode of malaria (p=0·595). The hazard rate for a malaria episode for high-tertile (cutoff 314·4 EU/mL) versus low-tertile (cutoff 143·6 EU/mL) in anticircumsporozoite titres after dose three (month 3) was 0·265 (95% CI 0·088–0·801; p=0·019), corresponding with a reduction in risk of clinical malaria of 73·5% (95% CI 20·0–91·2; figure 4).

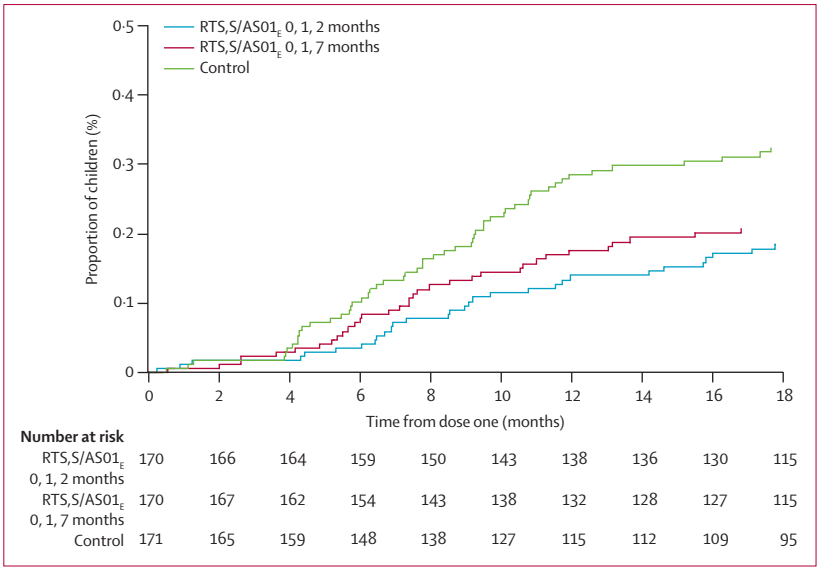

***Figure 3*: Incidence of at least one *Plasmodium falciparum* malaria episode (primary case definition) in the three study groups, during the whole study duration (total vaccinated cohort)**

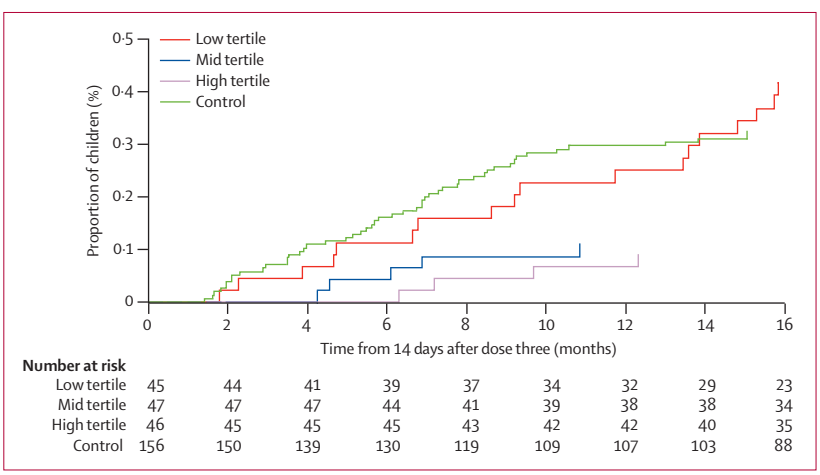

***Figure 4*: Incidence of *Plasmodium falciparum* malaria in RTS,S/AS01$_E$ 0, 1, 2 month group and the control group, presented by tertiles of anticircumsporozoite antibodies (according-to-protocol cohort for efficacy)**

Discussion

Vaccination of infants aged 6–10 weeks with RTS,S/AS01$_E$ according to a 0, 1, 2 month schedule given with EPI vaccines provides 52·5% vaccine efficacy against first malaria episodes and 59·1% vaccine efficacy against all malaria episodes (according-to-protocol analysis; table 3; panel). Occurrence of serious adverse events was similar in RTS,S/AS01$_E$ and control groups. A delayed third dose, as assessed with a 0, 1, 7 month immunisation schedule, did not improve anticircumsporozoite antibody immunogenicity or efficacy.

We investigated the safety, immunogenicity, and efficacy of RTS,S/AS01$_E$ given with a pentavalent vaccine containing a hepatitis-B-antigen component. We previously reported[12] that safety surveillance over 8 months showed no concerning imbalance of serious adverse events across

groups; no serious adverse event was judged to be related to study vaccination. Our 19-month data strengthen the reassuring safety assessment published previously: over the whole study period the proportion of serious adverse events was similar across groups, with none attributed to study vaccination, and no concern raised by the relative frequency of individual events across groups.

RTS,S/AS01$_E$ induced highly protective immune responses against hepatitis B, when given with hepatitis-B-containing EPI vaccines. At month 19, all recipients of RTS,S/AS01$_E$ were seroprotected against the hepatitis-B virus. Anti-HBsAg antibody GMTs were highest in the RTS,S/AS01$_E$ 0, 1, 7 month group and lowest in the control group. High RF1-like antibody responses were induced by giving RTS,S/AS01$_E$ with the pentavalent EPI vaccine.

In 2006, WHO and other representatives of the malaria-vaccine scientific community published the Malaria Vaccine Technology Road Map,[25] in which the first goal for 2015 was the development and licensure of a first-generation malaria vaccine that has a protective efficacy of more than 50% against severe malaria and death, and lasts longer than 1 year. The road map recognised that while the relation between vaccine effect on clinical disease and death is complicated, a vaccine that provides protection against clinical disease will provide an equivalent or higher protection against severe disease and death.[25]

Our trial was not designed to assess vaccine efficacy against severe malaria, but safety surveillance showed that *P falciparum* infections reported as a serious adverse event (hospitalisation with malaria) were reduced by about 50% in the RTS,S/AS01$_E$ 0, 1, 2 month group (11 episodes) compared with the control group (23 episodes). One case of severe malaria was reported in the RTS,S/AS01$_E$ 0, 1, 2 month group, three in the RTS,S/AS01$_E$ 0, 1, 7 month group, and five in the control group. In view of the formal efficacy endpoints of the study, the point estimate of vaccine efficacy of RTS,S/AS01$_E$ given at 0, 1, 2 months was 52·5% against the first or only episode of malaria (p=0·001) and 59·1% against all malaria episodes (p=0·0001), during 17 months after completion of vaccination. When considering data gathered after dose three, the 0, 1, 2 month and 0, 1, 7 month schedules had similar efficacy. However, during the whole study (months 0–19), the 0, 1, 7 month schedule had a lower point estimate of vaccine efficacy than did the 0, 1, 2 month schedule. This finding probably results from suboptimum protection between the second and third RTS,S/AS01$_E$ doses in the 0, 1, 7 month group, as suggested by the lower vaccine efficacy 6 months after three doses in the 0, 1, 2 month group than after two doses in the 0, 1, 7 month group. Overall, vaccine-efficacy results show that a third dose is important, and that its delay until measles immunisation at 9 months of age does not improve protection over 19 months of follow-up. These data are therefore supportive of the selection of a 0, 1, 2 month schedule for the continuing phase 3 assessment of RTS,S/AS01$_E$.

No immunological correlate of protection has been established for malaria. We showed an association between anticircumsporozoite-antibody titres and subsequent risk of malaria when assessing GMTs in the tertile analysis, but not the ten-times-increase analysis. Past studies have shown an association between antibody responses and protection against malaria infection, but not disease.[5,7,10,11,26] The reasons for these differences are not clear, but available information, including data presented here, show that the humoral response to the circumsporozoite protein is an important component, or marker, of protective immunity. Cellular immune responses also seem to play a part, as shown in the challenge model.[26] The study of cellular immune responses in vaccine efficacy studies in conditions of natural malaria transmission is in progress.

Our results are very encouraging, but the trial had an open design, which carries an inherent risk of observation bias. While procedures for malaria diagnosis were in place from the study start as part of the safety surveillance and provision of care, the plan to measure vaccine efficacy was introduced as a protocol amendment after the trial had started. Reassuringly, over 75% of efficacy data were gathered prospectively. The passive detection of malaria and serious adverse events relied on health-seeking behaviour, but access to care was facilitated for all participants. Point estimates of vaccine efficacy in the two assessed vaccine schedules suggests protection with the 0, 1, 2 month schedule was better than with the 0, 1, 7 month schedule, but no comparative statistical analysis was done because the trial was not powered for formal comparison between schedules. Observation in this trial stopped at 19 months; however, a full assessment of the effect of the malaria candidate

***Panel*: Research in context**

Systematic review

We searched PubMed for reports published upto Jan 20, 2011 using the terms "malaria vaccines"[MeSH Terms] OR ("malaria"[All Fields] AND "vaccines"[All Fields]) OR "malaria vaccines"[All Fields] OR ("malaria"[All Fields] AND "vaccine"[All Fields]) OR "malaria vaccine"[All Fields]) AND efficacy[All Fields] AND ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields]; no language restrictions were applied. Apart from RTS,S-based vaccines, no other candidate vaccines have been shown to be protective against natural transmission of malaria in children. In those aged 5–17 months from Kenya and Tanzania, vaccine efficacy against clinical malaria disease of the RTS,S/AS01$_E$ candidate vaccine, detected by active or passive case detection, was 53% (95% CI 28–69; p=0·0005) during a mean follow-up of 8 months after dose three, 39% (20–54; p=0·0005) 12 months after dose three, and 46% (24–61; p=0·0004; Kenya data only) 15 months after dose three.[7,24] When an RTS,S-based vaccine, in this case with the AS02 adjuvant, was given to infants together with EPI vaccines for the first time in Tanzania, vaccine efficacy against malaria detected by active detection of infection was 65% (21–85; p=0·01) 6 months after dose three.[11]

Interpretation

RTS,S/AS01$_E$ candidate vaccine protects infants against malaria, which for the 0, 1, 2 month vaccination schedule was consistent with WHO's Malaria Vaccine Technology Road Map for licensure of a first-generation malaria vaccine.[25] The 0, 1, 2 month schedule has been selected for phase 3 candidate vaccine evaluation.

vaccine will need longer follow-up. A continuing phase 3, observer-masked, trial designed to assess vaccine efficacy against various endpoints relevant to public health, including severe malaria, anaemia, and mortality, with a planned follow-up of several years will allow a more thorough assessment of the malaria vaccine candidate (NCT00866619).

We showed similar rates of serious adverse events for RTS,S/AS01$_E$ given together with EPI vaccines compared with EPI vaccines alone, during 19 months follow-up. Anticircumsporozoite antibody GMTs at month 19 remained significantly higher in RTS,S/AS01$_E$ recipients than in controls. Antibody responses in the 0, 1, 2 month schedule were associated with protection against malaria, which was consistent with WHO's Malaria Vaccine Technology Road Map target for a first-generation malaria vaccine.[25]

Contributiors

KPA, SAb, SAg, JV, AL, EJ, ML, M-CD, BS, CL, DL, BG, JC, PK, SOA, MT, and BL conceived and designed the experiments. KPA, SAb, SAg, JL, SS, RO, MS, NS, JFF, DD, MC, SI, KO-K, MP, TM, SAp, GM, MT, EK, RM, ABH, OB, KK, GA, DC, PK, SOA, MT, and BL undertook the experiments. KPA, SAb, JV, AL, EJ, ML, BS, DL, BG, JC, SOA, MT, and BL analysed the data. EJ, DD, ML, TC, PV, and MS contributed reagents, materials, and analytical devices. KPA, JV, ML, AL, and BG wrote the report.

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Conflicts of interest

JV, AL, EJ, ML, M-CD, DL, and JC are employees of GlaxoSmithKline Biologicals. JV, AL, M-CD, and JC own shares in GlaxoSmithKline. JC was listed as inventor of patented malaria vaccines, including RTS,S. TC, PV, MS, BS, and CL are employees of Malaria Vaccine Initiative, which supports the development and testing of several malaria vaccines. MT and BG have grants from Malaria Vaccine Initiative pending. All other authors declared no conflicts of interest.

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