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

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Summary

Background **Malaria remains a leading global health problem that requires the improved use of existing interventions and the accelerated development of new control methods. We aimed to assess the safety, immunogenicity, and initial efficacy of the malaria vaccine RTS,S/AS02D in infants in Africa.**

Methods **We did a phase I/IIb double-blind randomised trial of 214 infants in Mozambique. Infants were randomly assigned to receive three doses either of RTS,S/AS02D or the hepatitis B vaccine Engerix-B at ages 10 weeks, 14 weeks, and 18 weeks of age, as well as routine immunisation vaccines given at 8, 12, and 16 weeks of age. The primary endpoint was safety of the RTS,S/AS02D during the first 6 months of the study, and analysis was by intention to treat. Secondary endpoints included immunogenicity and analysis of new *Plasmodium falciparum* infections during a 3-month follow up after the third dose. Time to new infections in the per-protocol cohort were compared between groups using Cox regression models. This study is registered with ClinicalTrials.gov, number NCT00197028.**

Findings **There were 17 children (15.9%; 95% CI 9.5–24.2) with serious adverse events in each group. In the follow-up which ended on March 6, 2007, there were 31 serious adverse events in the RTS,S/AS02D group and 30 serious adverse events in the Engerix-B group, none of which were reported as related to vaccination. There were four deaths during this same follow-up period; all of them after the active detection of infection period had finished at study month 6 (two in RTSS/AS02D group and two in the Engerix-B group). RTS,S/AS02D induced high titres of anti-circumsporozoite antibodies. 68 first or only *P falciparum* infections were documented: 22 in the RTS,S/AS02D group and 46 in the control group. The adjusted vaccine efficacy was 65.9% (95% CI 42.6–79.8%, p<0.0001).**

Interpretation **The RTS,S/AS02D malaria vaccine was safe, well tolerated, and immunogenic in young infants. These findings set the stage for expanded phase III efficacy studies to confirm vaccine efficacy against clinical malaria disease.**

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Introduction

Malaria remains a leading global health problem, and control of the disease will need the improved use of existing interventions and the accelerated development of new control methods. The malaria vaccine candidate RTS,S (GlaxoSmithKline, Rixensart, Belgium), formulated with the adjuvant system AS02 or AS01, specifically targets the pre-erythrocytic stage of *Plasmodium falciparum*, and has been shown to confer complete or partial protection against experimental infection.[1,2] Short-term protection against infection was shown in immunised adult men in The Gambia in 1998.[3] In 2004, we reported a proof of concept study in African children aged 1 to 4 years, which established that RTS,S reduced the risk of *P falciparum* infection, uncomplicated malaria, and severe disease, and that this protection lasted for at least 18 months.[4,5]

The goal is to register RTS,S for use in infants and children living in *P falciparum* endemic locations. The plan has two main drivers: the need to protect the youngest age groups and to include RTS,S in the Expanded Program of Immunisation (EPI) scheme. In most areas with stable malaria transmission, children younger than 2 years have a large and disproportionate incidence of severe disease and death. There is growing recognition that malaria control strategies need to give top priority to protecting infants as soon as possible after birth.[6,7] The endemic countries of sub-Saharan Africa often have weak health systems. In this context, the EPI is the best functioning system of regular health contacts with infants, capable of delivering millions of doses across the continent including rural areas.[8]

The RTS,S clinical development plan involves several African research centres across Africa, doing trials that will generate information on the selection of the final formulation and schedule to be studied in a phase III trial for registration. In this paper, we report the results of the first evaluation of safety, immunogenicity, and efficacy test of concept of the RTS,S/AS02D vaccine formulation when administered to African infants living in a rural area of intense *P falciparum* transmission on a vaccination schedule staggered with the existing EPI schedule.

Methods

Study Design

The study was done at the Centro de Investigação em Saude de Manhiça (CISM, Manhiça Health Research

Centre) in Ilha Josina and Taninga (50 km north of Manhiça, Mozambique) between June, 2005, and March, 2007. Ilha Josina has been previously described.[4] Taninga faces Ilha Jossina directly across the Incomati River. The climate is subtropical with two distinct seasons including a warm and rainy season from November to April. Malaria transmission is perennial with some seasonality and mostly attributable to *P falciparum*. *Anopheles funestus* is the main vector. Amodiaquine plus sulphadoxine-pirimethamine was the first line treatment for uncomplicated malaria until September, 2006, when it was changed to artesunate plus sulphadoxine-pyrimethamine. Since 2005, indoor residual spraying, initially with carbamates (lambdacyhalothrin) but changed to dichloro-diphenyl-trichloroethane in December, 2006, has been implemented by the Mozambique Ministry of Health. In accordance with national recommendations, pregnant women at screening were provided with and trained to use an insecticide-treated bednet. Primary health posts and maternity units at the study sites were upgraded including 24-h care and transport to the Manhiça District Hospital.

This phase I/IIb double blind randomised trial assessed the safety, immunogenicity, and efficacy of RTS,S/AS02D when administered to infants at 10, 14, and 18 weeks of age. The protocol (NCT00197028, BB-IND 10514) was approved by the Mozambican National Bioethics Committee, the Hospital Clinic of Barcelona Ethics Review Committee, and the PATH Human Subjects Protection Committee, and was implemented according to the International Conference of Harmonization Good Clinical Practices guidelines. GlaxoSmithKline (GSK) monitored the study. A local Safety Monitor and a Data and Safety Monitoring Board oversaw the design, conduct, and results of the trial.

Participants

Informed consent was obtained from resident pregnant women in their third trimester. An information sheet was explained to groups of pregnant women and individual consent was obtained only after passing an oral comprehension test of the study information. Signature (or thumbprint if illiterate) were witnessed and countersigned by an impartial member of the community. Consenting women were counselled and screened for HIV (Determine HIV1-2, Abbott Laboratories, Tokyo, Japan, and UNI-GOLD HIV, Trinity Biotech, Bray, Ireland) and hepatitis B (Determine HBsAg, Abbott Laboratories, Abbott Park, IL, USA). HIV-positive women were referred to Manhiça District Hospital for management as per National Guidelines, including reduction of mother-to-child transmission and provision of antiretroviral therapy as indicated. Mothers with hepatitis B were counselled about the risk to their infants, and hepatitis B vaccine was offered for newborn babies.

Under separate maternal informed consent, infants were screened at between 6 and 12 weeks of age, including a medical history, examination, and blood sampling for baseline haematology, biochemistry, and immunology. Inclusion criteria included normal gestational period and absence of obvious medical abnormalities. Children of mothers who had hepatitis B or HIV, those not receiving oral poliovirus vaccine and Bacille Calmette-Guérin vaccine at least 1 week before study vaccination, and those receiving other pre-study vaccinations were excluded.

Photo identification was provided and included names of the child and mother and census identification number,[9] and a unique study number issued at the screening. The first infant was enrolled on Aug 23, 2005, and the last on Sept 12, 2006. Follow-up activities for the double-blind phase were completed on March 6, 2007, when the last recruited child reached their 6 month study visit.

Procedures

Figure 1 represents the trial design and follow-up scheme. Eligible children were enrolled the day of the first vaccination with DTPw/Hib (TETRActHib Aventis Pasteur) and randomly assigned to receive three doses of study vaccines (RTS,S/AS02D [GSK Biologicals, Rixensart, Belgium] or hepatitis B vaccine [Engerix-B, GSK Biologicals, Rixensart, Belgium]) staggered by 2 weeks with DTPw/Hib and oral poliovirus vaccines and

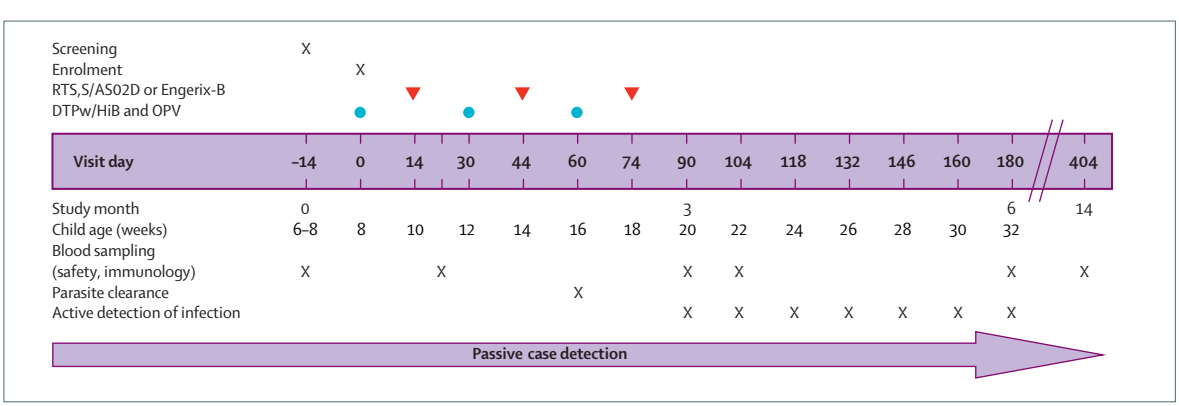

Figure 1: **Trial design**
DTPw=diphtheria-tetanus-whole-cell pertussis. Hib=*Haemophilus influenzae* type b. OPV=oral poliovirus vaccine.

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administered at 10, 14, and 18 weeks of age. Block (1:1 ratio, block size of 2) randomisation was done at GSK Biologicals (SAS version 8). The code was released once databases had been monitored, checked for inconsistencies, and locked.

The infants randomly assigned to malaria vaccine received RTS,S/AS02D (0·5 mL) containing 25 µg of RTS,S and the AS02D (previously described[10]). RTS,S is a hybrid recombinant protein consisting of the *P falciparum* circumsporozoite protein central tandem repeat and carboxy-terminal regions fused to the amino-terminus of the S antigen of hepatitis B virus (HBsAg). The proteins auto-assemble to form a particle that also includes unfused S antigen.

Vaccines were administered intramuscularly in the right (DTPw/Hib) or left (study vaccines) anterolateral thigh. To ensure that the study vaccines were delivered in a double-blind fashion, the preparation and vaccination procedures took place in separate limited-access rooms. Vaccine syringes were masked with opaque tape to prevent the mother from seeing the syringe contents. The vaccination team was not blinded but was not involved in any other study procedures. No other members of the trial team were aware of the which study vaccine any child received.

After each vaccination, infants were observed for an hour. Trained field workers visited the children at home every day for the next 6 days to record local and general adverse events. As described elsewhere,[11] unsolicited adverse events were recorded for 30 days after each dose, and serious adverse events were recorded throughout the study by use of the morbidity surveillance system based at the health facility. Haematological renal and hepatic function were assessed at several time points (figure 1). Anti-HBsAg antibody titres were measured at baseline and 1 month after the third dose of study vaccines. Anti-circumsporozoite antibody titres were measured at baseline, and at 1 and 3·5 months after the third dose of the study vaccines. Antibodies against diphtheria and tetanus toxins and polyribosyl ribitol phosphate for *Haemophilus influenzae* type b were measured 1 month after dose 3 of DTPw/Hib. Antibodies against *Bordetella pertussis,* were measured at baseline and 1 month after the third dose of DTPw/Hib.

Malaria infections by *P falciparum* were discovered by active detection and by passive case detection at health facilities in the study area. Active detection occurred at predefined intervals when a blood slide for parasitaemia determination was collected and the axillary temperature was recorded irrespective of symptoms. All children with positive slides were treated with first line treatment and excluded from further assessment of active detection of infection. In all children, parasitaemia was presumptively cleared with amodiaquine (10 mg/kg per day for 3 days) plus sulfadoxine (25 mg/kg) and pyrimethamine (1·25 mg/kg) 2 weeks before the final dose of RTS,S/AS02D or Engerix-B. Parasitaemia was checked at the time of dose 3 and, if present, treated with second-line treatment based on artemether-lumefantrine. Only children without parasitemia started the active detection of infection, commencing 2 weeks after the third dose and repeated every-other week for 12 weeks.

Passive case detection was done through monitoring of all attendances to health facilities and ascertainment of episodes of clinical malaria, as described in detail elsewhere.[4] All children with documented fever (37·5ºC or higher) or history of fever in the preceding 24 h, or pallor, had blood taken for parasite and packed-cell volume determinations. Children meeting admission criteria were referred to the Manhiça District Hospital for hospitalisation. Clinical management was provided according to standard national guidelines. On admission and discharge all relevant information was recorded on standardised forms.

Giemsa-stained blood slides were assessed using standard quality-controlled procedures described elsewhere.[4] Biochemical parameters were measured using a dry biochemistry photometer VITROS DT II (Orto Clinical Diagnostics, Johnson & Johnson Company, Rochester, NY, USA). Haematological tests were done with a Sysmex KX-21N cell counter (Sysmex Corporation Kobe, Japan). Packed-cell volume was measured in heparinised microcapillary tubes using a Hawksley haematocrit reader after centrifugation.

Antibodies against the circumsporozoite repeat region were measured by a standard ELISA, using plates absorbed with recombinant R32LR with an assay cutoff of 0·5 EU/mL. Anti-HBsAg antibody levels were measured with a commercial radioimmunoassay (AUSAB, Abbott, IL, USA) with an assay cutoff of 10 IU/mL. Anti-PRP antibodies were measured by ELISA with a cutoff of 0·15 µg/mL. Anti-diphtheria and anti-tetanus antibodies titres were measured by ELISA with an assay cutoff of 0·10 IU/mL. Anti-whole-cell-B pertussis antibody titres were determined by ELISA (Labsystems, Vantaa, Finland) with an assay cutoff of 15 EU/mL.

Statistical analysis

The analysis was based on a prospectively-defined report and analysis plan. The primary endpoint was safety of the RTS,S/AS02D during the first 6 months of the study. Secondary endpoints included immunogenicity analyses and estimation of vaccine efficacy. All children who received at least one dose of DTPw/Hib were included in the intention-to-treat safety analysis.

Anti-circumsporozoite and anti-HBsAg antibody data were summarised by geometric mean titres with 95% CIs. Anti-circumsporozoite seropositivity was defined as 0·5 EU/mL or more, whereas seroprotection from hepatitis B was defined as 10 IU/mL or more.

The test of concept for vaccine efficacy was done with a per protocol cohort, which included infants who met all eligibility criteria, completed the vaccination course, and contributed follow-up time during the active detection of

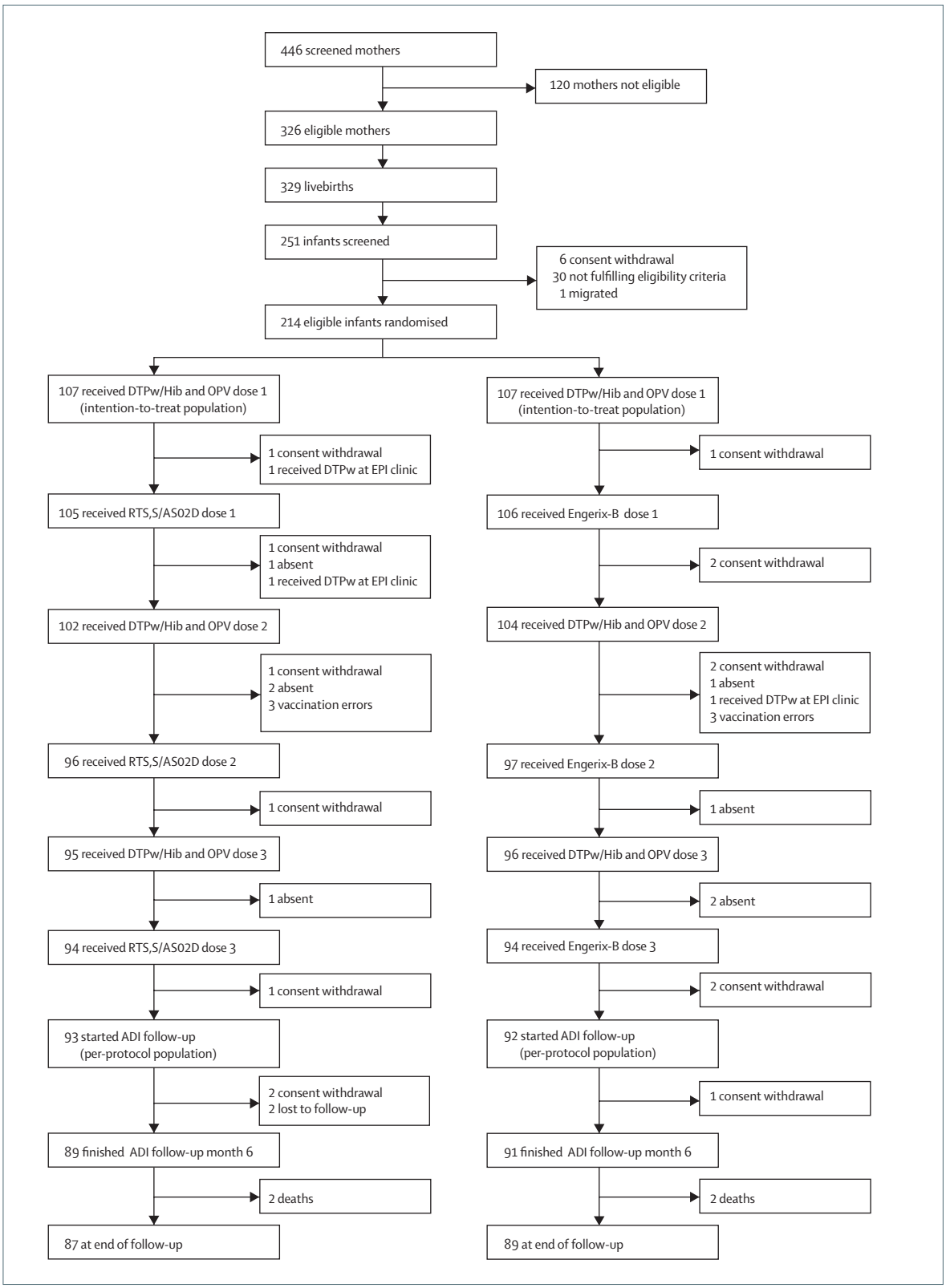

Figure 2: **Trial profile**
DTPW=diphtheria-tetanus -whole-cell-pertussis. Hib=*Haemophilus influenzae* type b. OPV=oral poliovirus vaccine. EPI=Expanded Program of Immunisation. ADI=active detection of infection.

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infection period. Cases were all first or only infections of asexual *P falciparum* detected during the follow-up period starting 14 days after dose 3 of RTS,S/AS02D or Engerix-B and ending with the visit at study month 6 (a roughly 3-month follow-up).

Further analyses of vaccine efficacy against clinical malaria were explored in an intention-to-treat cohort and included first or only episodes from the time of enrolment until the date on which the last enrolled infant completed their active detection of infection follow-up (March 6, 2007) and in the same per protocol cohort and follow-up period as in the efficacy assessment of new infections. The primary case definition for clinical malaria was fever (axillary temperature ≥37·5ºC) with an asexual parasitaemia of *P falciparum* of 500/µL. This definition has a reported sensitivity and specificity of greater than 90%[12]. Other exploratory efficacy analyses used secondary case definitions for clinical malaria including fever or history of fever in the previous 24 h plus any asexual *P falciparum* parasitaemia. Person years at risk was adjusted for absences from the study area and for antimalarial drug usage as previously described.[4]

Vaccine efficacy was defined as 1 minus the hazard ratio. Vaccine efficacy was adjusted by distance to health facility, calculated according to previously described methods,[4] and community of residence. The adjusted vaccine efficacy, assessed using Cox regression models, is reported throughout the text unless otherwise stated. The effect of anti-circumsporozoite antibody titres on the risk of malaria infection was assessed in the group who received RTS,S/AS02D by comparing the hazard ratio of infants with responses lower than the first tertile against those with responses higher than the second tertile, as well as estimating the hazard ratio per doubling of anti-circumsporozoite antibody titre with Cox regression models. Finally, we compared the geometric mean titre of children who had at least one episode of malaria infection against those without documented malaria infection using a Wilcoxon Rank Sum test.

The sample size was based on an assessment of vaccine safety. A trial with 100 participants in each group had an 80% power to detect a difference in the proportion of adverse events of 26% or more if the frequency of an event in the Engerix-B group was 10% or more. A trial of this size also had 90% power to detect an efficacy against malaria infection of 45% or more, assuming an attack rate of 75% or more in the control group over the surveillance period. Analyses were done with SAS 8 and STATA 9.

This study is registered with ClinicalTrials.gov, number NCT00197028.

Role of the funding source

GlaxoSmithKline and *Centro de Investigação em Saude de Manhiça* both received financial support from the PATH Malaria Vaccine Initiative (MVI), which was involved in all aspects of the study design and interpretation. MVI funded this work through a grant from the Bill & Melinda Gates Foundation. Core funding for CISM is provided by the Spanish Agency for International Cooperation (AECI). The corresponding author had full access to all the data in the study and had the final decision to submit for publication.

Results

214 screened children were enrolled in the trial and received the first dose of DTPw/Hib vaccine (figure 2). Baseline characteristics for both groups are presented in table 1.

Figure 3 shows the proportion of all administered doses where a solicited symptom was recorded. The figure presents four groups, since the data for DTPw/Hib and oral poliovirus vaccine are segregated by randomisation assignment. The percentage of children with pain was high and similar in all groups. The relative percentage of symptoms was similar after each injection of RTS,S/AS02D and comparable in magnitude (data not shown). No grade-3 solicited symptom was documented in the RTS,S/AS02D group, and the incidence was low in the other groups (data not shown). The number of adverse events after DTPw/Hib vaccine or after study vaccines were similar in both groups (data not shown). In the intention-to-treat cohort, which included all children who had received at least one vaccine dose and who were followed-up until 6 months after the first dose, there were 17 children (15·9%; 95% CI 9·5–24·2) with serious adverse events in each group. In the follow-up which ended on March 6, 2007, there were 31 serious adverse events in the RTS,S/AS02D group and 30 serious adverse events in the Engerix-B group, none of which were reported as related to vaccination. There were four deaths during this same follow-up period; all of them after the active detection of infection period had finished at study month 6 (two in RTSS/AS02D group and two in the Engerix-B group). All of the deaths were at home. Verbal autopsies suggested that one death in the RTS,S/AS02D group

	Engerix-B (n=92)	RTS,S/AS02D (n=93)
Age		
Age at first dose (weeks)	8·3 (1·0)	8·3 (1·4)
Sex		
Girl	53 (58%)	43 (46%)
Boy	39 (42%)	50 (54%)
Community		
Ilha Josina	64 (70%)	63 (68%)
Taninga	28 (30%)	30 (32%)
Distance (km)		
0–5	79 (86%)	77 (83%)
5–10	9 (10%)	9 (10%)
10–17	4 (4%)	7 (7%)

Data are mean (SD) or number of children (%).

Table 1: **Baseline characteristics**

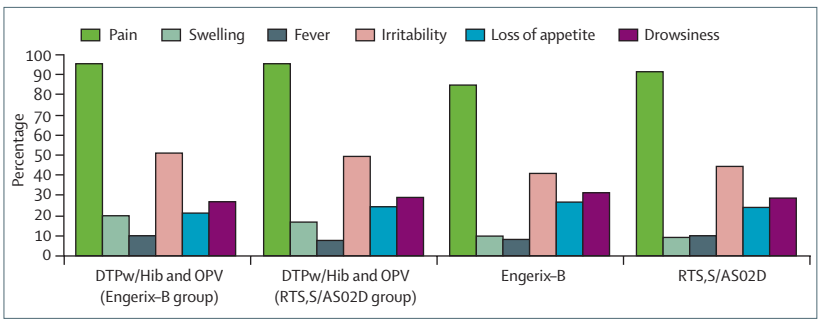

Figure 3: **Percentage of doses with solicited general symptoms reported during the 7 days after vaccination**
DTPw=diphtheria-tetanus-whole-cell pertussis. Hib=*Haemophilus influenzae* type b. OPV=oral poliovirus vaccine.

	Engerix-B		RTS,S/AS02D	
	n	value (95% CI)	n	value (95% CI)
Anti-circumsporozoite				
Baseline	77	0·4 (0·3–0·4)	76	0·4 (0·3–0·5)
30 days after third dose	68	0·3 (0·2–0·3)	71	199·9 (150·9–264·7)
106 days after third dose	61	0·4 (0·3–0·5)	53	58·8 (41·8–82·8)
Anti-HBsAg				
Baseline	70	16·6 (11–25)	72	14 (9·6–20·5)
30 days after third dose	64	392·4 (297–518·5)	68	10 081·6 (7394·9–13 744·4)

Table 2: **Geometric mean titres for Anti-CS and Anti-HBsAg**

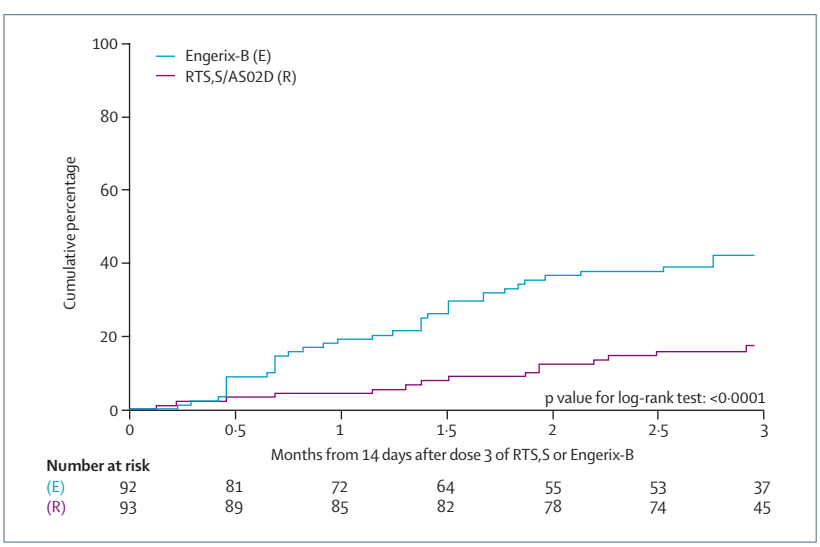

Figure 4: **Kaplan-Meier curves showing the cumulative percentage of participants with at least one episode of malaria infection**

was due to septic shock, and the remaining three due to gastroenteritis and severe dehydration. The values and proportion of abnormal haematology and biological values after the first dose and third dose were similar in both groups and did not seem suspicious (data not shown).

For the 214 infants enrolled in the trial, data for the EPI antigens responses are available for 151 participants after dose 3 (76 in the RTS,S/AS02D and 75 in the control group). There were no differences in seroprotection, seropositivity, and titres between the infants in the two groups. All but three children reached seroprotective concentrations against all EPI antigens. These three children will be re-immunised with the antigens to which they have failed to respond.

Anti-circumsporozoite and anti-HBsAg antibody levels measured against circumsporozoite and HBsAg are shown in table 2. At screening, 24 (32%) of 76 infants in the RTS,S/AS02D group and 26 (34%) of 77 of infants in the Engerix-B group had low titres of detectable anti-circumsporozoite antibodies. A month after dose 3, 99% (70 of 71) of infants who received RTS,S/AS02D had detectable anti-circumsporozoite antibodies, whereas the corresponding figure for infants who received Engerix-B was 4% (3 of 68). 14 weeks after dose 3 (study month 6), the proportion of anti-circumsporozoite positives in the RTS,S/AS02D remained high (98%) but the geometric mean titre had decreased. The anti-circumsporozoite geometric mean titre in the Engerix-B group remained low, even though the prevalence of detectable antibodies increased to 20% (12 of 61).

Figure 4 shows the proportion of children with at least one episode of malaria infection during the active detection of infection follow-up starting 14 days after dose 3 of RTS,S/AS02D or Engerix-B up to study month 6. A total of 68 new infections were documented during this follow-up period, 22 in the RTS,S/AS02D group and 46 in the Engerix-B group. The crude vaccine efficacy estimate was 62·2% (95% CI 37·1%–77·3%, p=0·0002) over the 3-month follow-up period. Adjusted by distance to the health centre and community of residence, the vaccine efficacy was 65·9% (42·6%–79·8%, p<0·0001; table 3). The point prevalence of infection at study month 6 was similar between the two groups (5% in the RTS,S/AS02D group *vs* 8% in the control group, p=0·536), nor were there differences between mean parasite densities (2082 parasites per mL (SD 5604) in the RTS,S/AS02D group versus 2579 (6088) in the control group, p=0·85).

Exploratory endpoints contained in the report and analysis included efficacy estimates for clinical malaria using different cohorts and case definitions. Efficacy based on an intention-to-treat cohort followed from month 0 to month 6, with the primary case definition of malaria (first or only episode of fever with more than 500 parasites per mL) detected through both active detection of infection and passive case detection, was 35·5% (95% CI –7·5% to 61·3%, p=0·093). Further efficacy estimates for clinical malaria in a per protocol cohort starting 14 days after dose 3 of RTS,S/AS02D or Engerix-B up to study visit at 6 months, the same follow-up period used in the primary vaccine efficacy estimate for infection, are shown in table 3.

The relation of anti-circumsporozoite antibody titres to the risk of malaria was examined in several ways. Firstly, we compared anti-circumsporozoite antibody titres after dose 3 of RTS,S/AS02D or Engerix-B in the group of infants where no malaria infection was documented during the follow-up versus those that had at least one

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	Engerix-B			RTS,S/AS02D			Crude vaccine efficacy		Adjusted vaccine efficacy	
	Events	PYAR	Rate	Events	PYAR	Rate	Efficacy (95%CI)	p	Efficacy (95%CI)	p
Malaria infection										
First or only episode of parasitaemia >0	46	17·2	2·7	22	21·8	1·0	62·2% (37·1%–77·3%)	0·0002	65·9% (42·6%–79·8%)	<0·0001
Clinical malaria										
First or only episode of fever and parasitaemia >500 per µL	22	19·6	1·1	9	22·6	0·4	64·4% (22·6%–83·6%)	0·009	65·8% (25·3%–84·4%)	0·007
First or only episode of fever or history of fever and parasitaemia >0	35	18·2	1·9	17	22·4	0·8	61·0% (30·2%–78·2%)	0·002	63·1% (33·6%–79·6%)	0·0009

PYAR= Person-years at risk. Rate=event/PYAR. Vaccine efficacy estimates adjusted by distance from health facility and community.

Table 3: **Vaccine efficacy from 14 days after third dose (month 3) of Engerix-B or RTS,S/AS02D until visit at month 6**

episode. On average anti-circumsporozoite antibody titres were higher in the group without malaria infection (208 *vs* 132, p=0·026). Secondly, the hazard ratio was 71% lower for infants in the higher tertile of the distribution of antibodies than for infants in the lower tertile (95% CI 8·4%–90·7%, p=0·035). Finally, we examined risk of malaria infection in relation to the increase in antibody titres. Doubling of antibody titres was associated with a reduction in the risk of a new infection of 6·4% (10·8–1·8, p=0·007). A ten times increase in anti-circumsporozoite titres is associated with a 19·8% reduction in the risk of new infections (31·6–5·9).

Discussion

The RTS,S/AS02D vaccine had a good safety profile and was well tolerated. Its reactogenicity did not increase with repeated doses and was indistinguishable to that of the licensed EPI control vaccines. There were no related serious adverse events, nor an imbalance in unsolicited adverse events between both groups. These are encouraging results for the use of the RTS,S candidate and this adjuvant system in infants. The need for an initial assessment of the safety, immunogenicity, and efficacy of the vaccine, in the absence of potentially confounding interactions with other antigens, led to this staggered design in relation to routinely administered EPI vaccines.

Around 34% of young infants living in this area of high transmission had detectable, albeit low, concentrations of anti-circumsporozoite antibodies at screening, probably transplacentaly transferred. In the control group, anti-circumsporozoite specific antibodies remained low over the first 8 months of life in the face of substantial malaria transmission. In infants immunised with RTS,S/AS02D, anti-circumsporozoite antibody responses were similar to those measured in older children (1–4 years of age),[4] suggesting that the anti-circumsporozoite responses were not significantly modified either by the presence of maternally-transferred antibodies or by the young age of these infants at the time of immunisation. Anti-hepatitis B antibody responses in the RTS,S/AS02D group were also consistent with those induced by this vaccine in the older children. The RTS,S/AS02D vaccine or the control were administered at least 14 days apart from the EPI vaccines to keep the risk of interference and confounded safety data to a minimum. Indeed we note that responses to EPI antigens were similar in both groups (data not shown).

The trial was designed mainly to assess safety. However, in view of the high intensity of transmission in this area, the trial also had sufficient power to assess efficacy against new infections, a biologically relevant endpoint. Consistent with the idea that pre-erythrocytic vaccines should act through their ability to neutralise sporozoites and limit the number of infected hepatocytes or liver stage merozoites that enter the blood stream, we have previously shown that delay of time to a new infection translates to reductions in both mild and severe malaria disease.[4]

Vaccine efficacy for new infections was 65% over a 3-month follow-up after completion of immunisations. This efficacy estimate is higher than the 45% reduction reported in a previous trial in older children aged 1 to 4 years, who were followed-up in a similar system of active detection in the same study area.[4] Nonetheless, caution should be exercised in the interpretation of this higher efficacy in younger infants. Firstly, the follow-up periods were not identical, being slightly shorter in the infants than in the older children. Secondly, confidence intervals of the two estimates overlap, and the difference could be due to chance. The prevalence of infection at the end of follow-up was lower in the RTS,S group than in the controls (5% *vs* 8%), but this difference is not significant. This low prevalence of infection is in sharp contrast to the high incidence of infection over this same period (around 50%). Prevalence is a function of the incidence and the duration of an infection. In the case of these children, the low prevalence and the lack of power to detect a difference is probably the result of the intense follow up, which included regular screening of infection and prompt treatment. Vaccine efficacy against infection reported here and previously in older children[4] was more robust that was seen in malaria-immune adults in The Gambia, and the much higher antibody concentrations against circumsporozoite in young children than in adults might contribute to these findings.

Efficacy against clinical malaria was also suggested by the exploratory analyses, but, again, these positive results should be viewed with some caution. Firstly, the study was not powered to assess efficacy against a clinical endpoint, but data were collected because they were relevant to our assessment of safety. Secondly, a cohort that is being actively followed up for detection of new infections implies repeated measurements and treatments that are likely to modify the natural course of an infection and its progression to clinical disease, rendering the efficacy estimates for this endpoint of uncertain validity. The previous proof of concept trial in older children used separate cohorts to assess efficacy against infection and clinical endpoints in order to avoid the introduction of bias, and it was in this context of separate cohorts that similar efficacy estimates were seen for reductions in new infections and clinical disease. Nonetheless, this infant study has produced encouraging estimates of efficacy against disease. Indeed, many of the clinical episodes were detected in the context of the active detection of infection visits. Considering the lack of acquired malaria immunity, infants became sick at low parasite densities,[13] possibly explaining the similar levels of vaccine efficacy documented against infection and disease over the same follow-up periods.

This trial was undertaken in an area of high transmission, but in the context of renewed and intense malaria control activities. All study participants were provided with free insecticide-treated bednets, two rounds of indoor residual spraying were done, with high coverage of the homes of study participants, and artemisinin combination therapies were introduced as first-line treatment for malaria. The future use and deployment of a malaria vaccine should be seen in the context of comprehensive malaria control programmes. This test of concept trial suggests that vaccine-induced protection adds to the protection attributable to other control methods, particularly vector control methods. Hypothetically, synergism between the vaccine and vector control methods could take place through a reduction in the number of infective bites, which results in spacing out challenges, thus allowing for improved immune responses,[14] and potentially through reductions in the infective innoculum of injected sporozoites which need to be controlled through vaccine-acquired immunity.

A frequent strategy in the process of antigen selection for inclusion in candidate vaccines has been the use of seroepidemiological data and its relation to infection or disease. Positive associations between certain immune responses induced through natural exposure and a reduced risk of disease have often been assumed deterministic of protection. We believe that immunological determinants of protection will best be established in the context of vaccine-induced efficacy. This infant study provides evidence of a strong association between vaccine-induced anti-circumsporozoite antibodies and a reduction in the risk of infection. This is of importance because up until now, immunogenicity was a marker of a response with no clearly proven relation to protection, which in turn could only be established through a clinical trial. This finding needs to be corroborated further in other trials, but this observation might be important in the clinical development plan of this vaccine.

The RTS,S/AS02D candidate malaria vaccine was safe, well tolerated, and highly immunogenic in young infants living in a rural area of southern Mozambique. These encouraging data need to be substantiated in phase III trials, but we have shown that a vaccine can reduce the risk of malaria infection in young African infants exposed to intense transmission of *P falciparum*. These results further strengthen the vision that a vaccine that can partly protect young African children and infants might contribute to the reduction of the intolerable burden of disease and death caused by malaria.

Contributors

All authors participated in the design, implementation, analysis, and interpretation of the study. PA was the principal investigator for the trial and coordinated the malaria vaccine teams in Manhiça and Barcelona. JA, PA, and MR led the implementation team, and together with PA were involved in all phases of the study and led the write up of the manuscript, which all other authors contributed to. AL led the clinical team at GlaxoSmithKline (GSK). JJA and ML led the data analysis. QB, JS, BS, and EM were heavily involved in field and hospital activities, and safety surveillance. MS and SL were the project managers. M-CD is the malaria vaccine project manager at GSK. M-AD coordinated the immunology read-out team. Inacio Mandomando, MNM and AB coordinated all laboratory work at CISM. JV was responsible for safety at GSK. JC and RB had had a key involvement in the design, implementation, and interpretation of this trial and the malaria Vaccine Program at GSK. BS provided key support throught the trial. JGMc is Research Director at MVI.

Conflict of interest statement

MVI supports the development and testing of several malaria vaccines. AL, ML, JV, M-AD, M-CD, WRB, and JC are employees of GSK Biologicals. AL, M-CD, WRB, and JC own shares in GSK. JC and WRB are listed as the inventors of patented malaria vaccines; however, neither individual holds a patent for a malaria vaccine. None of the other authors declare any conflict of interest.

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