



Efficacy of RTS,S/AS01E malaria vaccine and exploratory analysis on anti-circumsporozoite antibody titres and protection in children aged 5–17 months in Kenya and Tanzania: a randomised controlled trial

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Summary

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Background RTS,S/AS01E is the lead candidate malaria vaccine. We recently showed efficacy against clinical falciparum malaria in 5–17 month old children, during an average of 8 months follow-up. We aimed to assess the efficacy of RTS,S/AS01E during 15 months of follow-up.

Methods Between March, 2007, and October, 2008, we enrolled healthy children aged 5–17 months in Kilifi, Kenya, and Korogwe, Tanzania. Computer-generated block randomisation was used to randomly assign participants (1:1) to receive three doses (at month 0, 1, and 2) of either RTS,S/AS01E or human diploid-cell rabies vaccine. The primary endpoint was time to first clinical malaria episode, defined as the presence of fever (temperature $\geq 37.5^{\circ}\text{C}$) and a *Plasmodium falciparum* density of 2500/ μL or more. Follow-up was 12 months for children from Korogwe and 15 months for children from Kilifi. Primary analysis was per protocol. In a post-hoc modelling analysis we characterised the associations between anti-circumsporozoite antibodies and protection against clinical malaria episodes. This study is registered with ClinicalTrials.gov, number NCT00380393.

Findings 894 children were assigned, 447 in each treatment group. In the per-protocol analysis, 82 of 415 children in the RTS,S/AS01E group and 125 of 420 in the rabies vaccine group had first or only clinical malaria episode by 12 months, vaccine efficacy 39.2% (95% CI 19.5–54.1, $p=0.0005$). At 15 months follow-up, 58 of 209 children in the RTS,S/AS01E group and 85 of 206 in the rabies vaccine group had first or only clinical malaria episode, vaccine efficacy 45.8% (24.1–61.3, $p=0.0004$). At 12 months after the third dose, anti-circumsporozoite antibody titre data were available for 390 children in the RTS,S/AS01E group and 391 in the rabies group. A mean of 15 months (range 12–18 months) data were available for 172 children in the RTS,S/AS01E group and 155 in the rabies group. These titres at 1 month after the third dose were not associated with protection, but titres at 6.5 months were. The level of protection increased abruptly over a narrow range of antibody concentrations. The most common adverse events were pneumonia, febrile convulsion, gastroenteritis, and *P falciparum* malaria.

Interpretation RTS,S/AS01E confers sustained efficacy for at least 15 months and shows promise as a potential public health intervention against childhood malaria in malaria endemic countries.

Funding PATH Malaria Vaccine Initiative (MVI), GlaxoSmithKline.

Introduction

Worldwide, mortality and morbidity from malaria are high.^{1,2} Interventions such as insecticide-treated bednets and highly effective artemisinin combination therapy have reduced malaria transmission in some areas.^{3–5} However, an effective malaria vaccine would be an important addition to these control strategies.

RTS,S (GlaxoSmithKline, Rixensart, Belgium) is a recombinant antigen that consists of circumsporozoite protein fused to the hepatitis B surface antigen (HBsAg). RTS,S has been formulated with two different adjuvant systems (one with an oil-in-water emulsion [AS02] and the other with liposomes [AS01]), which contain the immunostimulants MPL and QS21.

Data from the first 8 months of this trial of RTS,S/AS01E showed efficacy of 53% (95% CI, 28–69, $p<0.0002$) against clinical falciparum malaria in children in Kenya and Tanzania.⁶ Efficacy data for an alternative RTS,S formulation, RTS,S/AS02A, were 29.9% (95% CI 11.0–44.8%, $p=0.004$) against clinical malaria for the first 6 months,⁷ and 35.3% (95% CI 21.6–46.6%, $p<0.0001$) during 18 months follow-up.⁸ RTS,S/AS01E is more immunogenic than RTS,S/AS02A^{9–11} and has entered phase 3 trials in seven African countries, and so the longevity of protection for this candidate vaccine needs to be assessed.

Antibodies to the circumsporozoite protein are protective in animals,¹² and in studies of infection in challenge models.⁹ Field trials show a relation between

anti-circumsporozoite antibody titres and re-infection rates after curative treatment with antimalarials.^{13,14} However, no association between anti-circumsporozoite antibody titres and clinical malaria has been identified.^{7,13}

We aim to assess the efficacy of RTS,S/AS01E during 15 months of follow-up after vaccination, and we present an exploratory analysis of vaccine efficacy in relation to antibody titres.

Methods

Participants

We did a randomised, controlled trial to assess the efficacy and safety of the RTS,S/AS01E malaria vaccine in children aged 5–17 months in Kilifi, Kenya, and Korogwe, Tanzania, as previously described.⁶ At screening, medical history and physical examination were done and blood samples were taken for haematological and biochemical tests. Participants were excluded from the trial if they had acute or serious disease at enrolment, a history of allergic reactions, a history of a previous blood transfusion, or a medical disorder not permitted by the protocol (eg, a weight-for-age Z score of less than –3 or other clinical signs of malnutrition at screening, major congenital defects, or a confirmed or suspected immunosuppressive or immunodeficient disorder).

Parents or guardians of all participants provided written informed consent with approved Swahili or Giriama consent forms. Parents or guardians who were illiterate thumbprinted the consent form, which was countersigned by an independent, literate witness. The study was approved by the Kenya Medical Research Institute National Ethics Committee, the National Institute for Medical Research of Tanzania, the Oxford Tropical Research Ethics Committee, the London School of Hygiene and Tropical Medicine Ethics Committee, and the Western Institutional Review Board in Seattle, WA, USA. The study was overseen by an independent data monitoring committee and local safety monitors, and done in accordance with the Helsinki Declaration of 1964 (revised 1996) and Good Clinical Practice guidelines.

Procedures

Children were randomly assigned (1:1) to receive either RTS,S/AS01E or human diploid-cell rabies vaccine (Sanofi Pasteur, Swiftwater, PA, USA). Participants were vaccinated between March, 2007, and August, 2007. Participants received three doses of vaccine intramuscularly at the left deltoid: dose one at enrolment, dose two 1 month after enrolment, and dose three 2 months after enrolment. Participants were enrolled by study clinicians and vaccinations were given by study nurses. Active case detection was done by fieldworkers through weekly household visits. Passive case detection was done by personnel at local dispensaries. Blood samples were taken at five points during the trial for serological studies: before vaccination; 1 month after dose three; in March, 2008 (4–10 months after dose three, mean 8 months); 12 months

after dose three; and in October, 2008 (12–18 months after dose three, mean 15 months).

Antibodies to the *Plasmodium falciparum* circumsporozoite repeat region were measured by ELISA at the Centre for Vaccinology (CEVAC; Ghent, Belgium) and results were reported in EU/mL. Plates were adsorbed with the recombinant antigen R32LR that contains the sequence [NVDP(NANP)15]2LR.¹⁵ Antibodies to HBsAg were measured with a commercial ELISA kit at GlaxoSmithKline Biologicals. HBsAg seroprotection rates were defined as the proportion of children who developed HBsAg antibody titres of 10 mIU/mL or more after vaccination.

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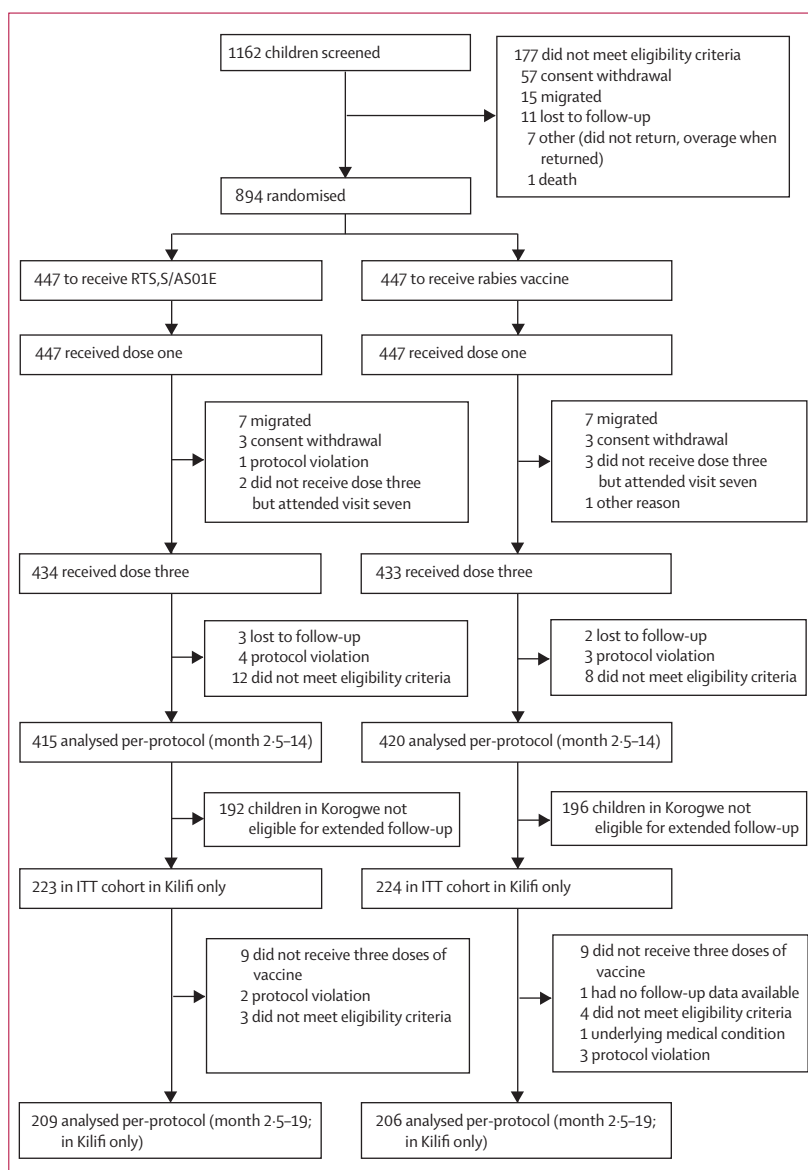


Figure 1: Consort diagram (total vaccinated cohort)

"Other" includes children missing vaccinations because of hospital admission, with contraindications to further vaccination, medical conditions not permitted by the protocol, and with no concomitant vaccination documentation. ITT=intention to treat.

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The primary endpoint was first or only clinical malaria episodes, defined as the presence of fever (temperature $\geq 37.5^{\circ}\text{C}$) and a *P. falciparum* density of 2500/ μL or more. The secondary case definition was fever with any falciparum parasitaemia.

Randomisation and masking

The RTS,S/AS01E and rabies vaccines were packaged in identical boxes labelled with treatment numbers from a randomisation sequence, which was computer generated by the study sponsor in blocks of six and stratified by study site. Participants were assigned treatment numbers on attendance to the dispensary. The study nurses who gave the vaccinations were unmasked to treatment, but the investigators, study participants, and parents or guardians of study participants were masked to treatment assignment.

The double-blind phase was completed after an average of 8 months follow-up, after which the investigators were unmasked to analyse data for the first 8 months.⁶ During the double-blind phase, the study database was maintained by a statistician at GlaxoSmithKline Biologicals. The single-blind phase (parents and guardians of trial participants remained masked) continued in both sites (Kilifi, Kenya, and Korogwe, Tanzania) until 12 months after dose three, and until October, 2008, in Kilifi, Kenya (the study site in Korogwe, Tanzania, did not have the infrastructure to sustain the extended follow-up).

Statistical analysis

On the basis of 90% power to detect a 30% efficacy rate at a significance level of 0.05, we calculated that 400 participants would be needed in each treatment group, assuming a projected malaria incidence of 36% in children that received the rabies vaccine.

The primary analysis was to estimate the efficacy of RTS,S/AS01E against first or only clinical malaria episodes that met the primary case definition on the per-protocol cohort (children who received all three vaccinations and for whom follow-up data were available). To examine the effect of the complete vaccination course, the per-protocol analysis began 2 weeks after the final vaccination. Secondary analyses estimated the efficacy of RTS,S/AS01E against multiple malaria episodes that met primary or secondary case definitions (on the per-protocol cohort). We also provide results of intention-to-treat analyses. Efficacy was defined as 1 minus the hazard ratio or rate ratio estimate of RTS,S/AS01E versus rabies vaccine group for Cox regression (first or only episode) or Poisson regression (multiple episodes). Estimates were adjusted for study centre, age at first vaccination, village, distance from the health facility, and use of insecticide-treated bednet. Differences in prevalence of asymptomatic parasitaemia were assessed by use of Fisher's exact test. To assess waning of efficacy we inspected the Kaplan-Meier plots for continued separation of the survival plots and tested the proportionality of hazards with Schoenfeld residuals and time-dependent Cox regression models.

Exploratory post-hoc analyses of log-transformed anti-circumsporozoite antibody titres were done on data from children in the per-protocol cohort who received the RTS,S/AS01E vaccine. Predicted anti-circumsporozoite antibody titres per week per child were calculated by fitting an exponential decay model. The relation between anti-circumsporozoite antibody titres and protection was assessed by Cox regression with anti-circumsporozoite antibody titres as a time-varying covariate in a survival model. We used an optimum two-group model, which was selected by varying the dichotomisation point to

| | RTS,S/AS01E | | | | Rabies vaccine | | | | Adjusted vaccine efficacy | | Vaccine efficacy | |
|---|-----------------------|--------------------|------|------------|-----------------------|--------------------|------|------------|---------------------------|---------|------------------|---------|
| | Number of individuals | Number of episodes | PYAR | Event rate | Number of individuals | Number of episodes | PYAR | Event rate | % (95% CI) | p value | % (95% CI) | p value |
| First or only episode, 12 months | | | | | | | | | | | | |
| ≥ 2500 parasites per μL | 415 | 82 | 367 | 0.22 | 420 | 125 | 351 | 0.36 | 39 (20-54) | 0.0005 | 38 (19-53) | <0.001 |
| >0 parasites per μL | 415 | 88 | 365 | 0.24 | 420 | 133 | 347 | 0.38 | 39 (20-53) | 0.0004 | 38 (19-53) | <0.001 |
| All episodes, 12 months | | | | | | | | | | | | |
| ≥ 2500 parasites per μL | 415 | 123 | 392 | 0.31 | 420 | 209 | 393 | 0.53 | 42 (22-57) | 0.0003 | 44 (23-59) | <0.001 |
| >0 parasites per μL | 415 | 138 | 391 | 0.35 | 420 | 238 | 392 | 0.61 | 44 (25-58) | 0.0001 | 45 (25-59) | <0.001 |
| First or only episode, 15 months | | | | | | | | | | | | |
| ≥ 2500 parasites per μL | 209 | 58 | 215 | 0.27 | 206 | 85 | 187 | 0.48 | 46 (24-61) | 0.0004 | 41 (18-58) | 0.002 |
| >0 parasites per μL | 209 | 63 | 213 | 0.3 | 206 | 91 | 183 | 0.5 | 46 (25-61) | 0.0002 | 41 (19-57) | 0.001 |
| All multiple episodes, 15 months | | | | | | | | | | | | |
| ≥ 2500 parasites per μL | 209 | 108 | 246 | 0.44 | 206 | 175 | 232 | 0.75 | 51 (29-66) | 0.0002 | 47 (22-63) | 0.001 |
| >0 parasites per μL | 209 | 122 | 246 | 0.5 | 206 | 201 | 231 | 0.87 | 52 (32-66) | 0.0001 | 47 (24-63) | <0.001 |

Per-protocol analysis is adjusted to account for use of insecticide-treated bednet, age, altitude, distance from the dispensary, and village. Analyses at 12 months for Kilifi and Korogwe. Analyses at 15 months for Kilifi only. PYAR=event rates per person year at risk.

Table 1: Per-protocol vaccine efficacy against *Plasmodium falciparum* clinical malaria episodes

maximise the log-likelihood that antibody titres would predict clinical outcome; bootstrapping was used to calculate CIs. A step function was then approximated by use of a cumulative normal distribution. We also explored other non-linear fits with power functions and fractional polynomials by use of the multiple fractional polynomial function on Stata 10.0, with $p < 0.1$ to retain additional power functions. This study is registered with ClinicalTrials.gov, number NCT00380393.

Role of the funding source

PATH Malaria Vaccine Initiative (MVI) funded the trial and was involved in all aspects of the trial. GlaxoSmithKline Biologicals was the study sponsor, and was involved in study design, data analysis, data interpretation, and writing of the study report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

894 children were enrolled (447 at each study site), and 447 were assigned to each group and followed up for 12 months (figure 1). 8 months' follow-up data have been reported previously.⁶ In the per-protocol analysis of 0–12 month follow-up, fewer children in the RTS,S/AS01E group had a first or only episode of clinical malaria meeting the primary case definition than did children in the rabies vaccine group ($p = 0.0005$; table 1). We recorded no evidence of waning efficacy on the survival plot (figure 2), or by Schoenfeld's residuals ($p = 0.63$). Similar results were obtained with the intention-to-treat analysis (table 2). 27 (7%) of 415 children who received RTS,S/AS01E and 49 (12%) of 420 who received rabies vaccine had more than one malaria episode in the per-protocol analysis, giving an adjusted vaccine efficacy against all clinical malaria episodes meeting the primary case definition of 42% (95% CI 22–57, $p = 0.0003$) by Poisson regression. Similar efficacy against multiple malaria episodes was found by intention-to-treat analysis (table 2).

In the per-protocol analysis for 0–15 month follow-up, fewer children in the RTS,S/AS01E group had a first or only episode of clinical malaria meeting the primary case definition than did children in the rabies vaccine group (table 1). We recorded no evidence of waning efficacy on the survival plot (figure 2), or by Schoenfeld's residuals ($p = 0.8$). Similar results were obtained with the intention-to-treat analysis (table 2). 58 (28%) of 209 of those who received RTS,S/AS01E and 85 (41%) of 206 of those who received the rabies vaccine had more than one malaria episode in the per-protocol analysis, giving an adjusted vaccine efficacy against all clinical malaria episodes meeting the primary case definition of 46% (95% CI 24–61, $p = 0.0004$) by Poisson regression. Similar efficacy against multiple malaria episodes was found by intention-to-treat analysis (table 2).

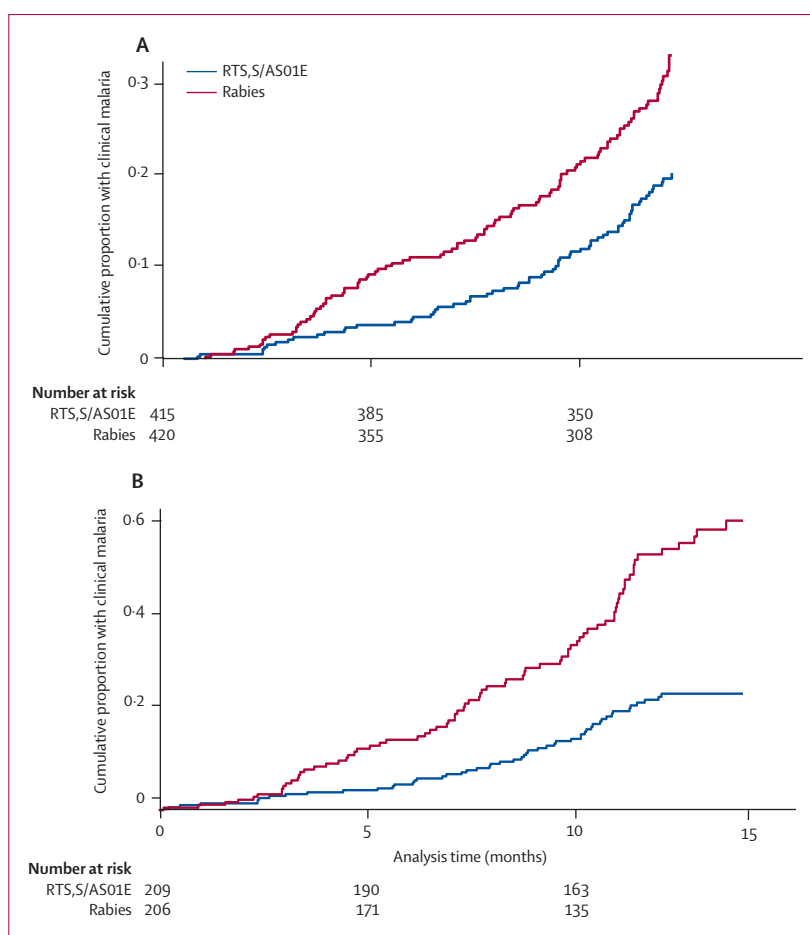


Figure 2: Kaplan-Meier curves showing the cumulative proportion of clinical falciparum malaria, by follow-up period (per-protocol cohort)

Clinical falciparum malaria defined as the presence of fever (temperature $\geq 37.5^{\circ}\text{C}$) and a *Plasmodium falciparum* density of $2500/\mu\text{L}$ or more. Kilifi and Korogwe cohorts over 12 months of follow-up; $p = 0.0005$ (A). Kilifi cohort only over 15 months of follow-up; $p = 0.0018$ (B).

Parasite prevalence was lower in children who received RTS,S/AS01E than in children who received rabies vaccine at 12 months (3.5% vs 8.2%, $p = 0.008$) and at 15 months (1.8% vs 7.5%, $p = 0.025$; data not shown). Mean haemoglobin concentrations were much the same in the two groups at 12 months (10.6 g/dL vs 10.5 g/dL, $p = 0.08$) and at 15 months (and 10.4 g/dL vs 10.3 g/dL, $p = 0.5$; data not shown).

Analyses were done on two cohorts: 894 children recruited in both Kilifi and Korogwe up to 12 months after dose three, and the children from Kilifi only for up to 18 months after dose three (mean 15 months, range 12–18 months). We recorded a significant increase in anti-circumsporozoite antibody titres 1 month after dose three in the RTS,S/AS01E group to a peak geometric mean titre of 539.6 EU/mL (95% CI 501–582; figure 3). In the RTS,S/AS01E group, antibody titres decreased during the trial; at 6.5 months the geometric mean titre was 71.9 EU/mL (95% CI 65.7–78.6), at month 14 the geometric mean titre was 41.6 EU/mL (95% CI 38–46),

| | RTS,S/AS01E | | | | Rabies vaccine | | | | Vaccine efficacy | |
|---|-----------------------|--------------------|------|------------|-----------------------|--------------------|------|------------|------------------|---------|
| | Number of individuals | Number of episodes | PYAR | Event rate | Number of individuals | Number of episodes | PYAR | Event rate | % (95% CI) | p value |
| First or only episode, 14 months | | | | | | | | | | |
| ≥2500 parasites per µL | 447 | 92 | 482 | 0.19 | 447 | 139 | 459 | 0.30 | 39 (20-53) | <0.001 |
| >0 parasites per µL | 447 | 101 | 477 | 0.21 | 447 | 147 | 454 | 0.32 | 36 (18-50) | <0.001 |
| All multiple episodes, 14 months | | | | | | | | | | |
| ≥2500 parasites per µL | 447 | 140 | 515 | 0.27 | 447 | 239 | 515 | 0.46 | 44 (24-58) | <0.001 |
| >0 parasites per µL | 447 | 162 | 514 | 0.32 | 447 | 272 | 514 | 0.53 | 43 (24-57) | <0.001 |
| First or only episode, 18 months | | | | | | | | | | |
| ≥2500 parasites per µL | 223 | 64 | 269 | 0.24 | 224 | 92 | 245 | 0.38 | 37 (14-55) | 0.004 |
| >0 parasites per µL | 223 | 72 | 264 | 0.27 | 224 | 98 | 240 | 0.41 | 34 (11-51) | 0.007 |
| All multiple episodes, 18 months | | | | | | | | | | |
| ≥2500 parasites per µL | 223 | 119 | 305 | 0.39 | 224 | 197 | 301 | 0.65 | 44 (20-61) | 0.002 |
| >0 parasites per µL | 223 | 137 | 305 | 0.45 | 224 | 226 | 300 | 0.75 | 43 (20-60) | 0.001 |

Analyses at 14 months for Kilifi and Korogwe. Analyses at 18 months for Kilifi only. PYAR=event rates per person year at risk.

Table 2: Intention-to-treat vaccine efficacy against *Plasmodium falciparum* clinical malaria episodes

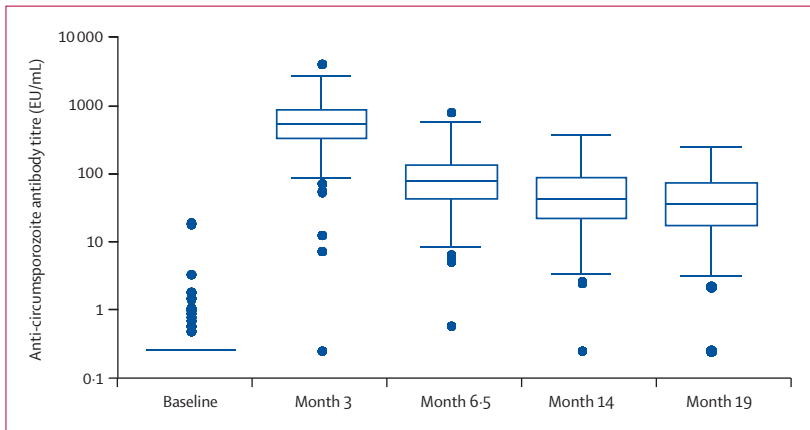


Figure 3: Anti-circumsporozoite antibody titres in individuals who received RTS,S/AS01E (per-protocol cohort)

and at month 19 the geometric mean titre was 31.3 EU/mL (26–38). Throughout the trial, anti-circumsporozoite antibody titres were higher in individuals in the RTS,S/AS01E group than they were in individuals in the rabies vaccine group, who had largely undetectable antibody titres throughout the study (data not shown).

Baseline HBsAg seroprotection rates (≥10 mIU/mL) were much the same in the two groups (roughly 95% of the participants). At 12 months, the seroprotection rates for HBsAg were higher in the RTS,S/AS01E group (99.7%, 95% CI 98.4–100) than in the rabies vaccine group (91.6%, 88.3–94.2). Mean geometric titres of HBsAg antibodies were 12 358.5 mIU/mL (10 707–14 264.8) in the RTS,S/AS01E group and 108.0 mIU/mL (91.8–127.1) in the rabies group (data not shown).

In the RTS,S/AS01E group, we noted no association between anti-circumsporozoite antibody titres at 1 month after dose three and protection from clinical malaria during 12 months of monitoring (hazard ratio [HR] for log increase in antibody titre 0.87, 95% CI 0.7–1.7, p=0.7; figure 4).

However, titres at month 6.5 showed an association with protection during 12 months (HR for log increase in anti-circumsporozoite antibody titre=0.43, 0.2–0.8, p=0.006). On examining the survival function by tertile of anti-circumsporozoite antibody titre, the lower tertile of responders seemed to be at increased risk compared with the upper two tertiles 6.5 months after vaccination (figure 4). The mean response in the lower tertile at month 6.5 was 26 EU/mL, compared with 225 EU/mL in the lower tertile 1 month after the third dose.

There were 139 serious adverse events reported during the 18-month follow-up, the most common of which were pneumonia, febrile convulsion, gastroenteritis, and *P. falciparum* malaria. The proportion of individuals who had at least one serious adverse event was lower in the RTS,S/AS01E group (51 of 447; 11.4%, 95% CI 8.6–14.7) than it was in the rabies group (88 of 447; 19.7%, CI 16.1–23.7). The 129 serious adverse events up to unmasking in August, 2008, have been reported elsewhere.⁶ A detailed report of safety and reactogenicity data has also been provided elsewhere.¹⁶

Our exponential model of antibody decay fit the data well (see webappendix p 1 for examples of individual fits). A linear fit between antibody titres and risk of clinical malaria was not significant (HR 0.68, 95% CI 0.45–1.03, p=0.07). The survival function plots by anti-circumsporozoite antibody tertiles showed that the increase in risk of clinical malaria was specific to the lower tertile of antibody titres at 6.5 months. We therefore reasoned that models that divide antibody titres into groups might fit the data better than would a linear model. We examined two-level models, dichotomising at a range of titres from 10 EU/mL to 350 EU/mL (webappendix p 3). An optimum fit (as measured by the log likelihood) was produced when the calculated antibody titres were dichotomised at 40 EU/mL (95% CI 32–59), with a hazard ratio of 0.52 (0.35–0.78, p=0.001) for the high titre group (data not shown).

See Online for webappendix

A two-level model implies an immediate change in risk of clinical malaria at a particular titre, which might be an oversimplification. To estimate how abrupt the change in risk was around 40 EU/mL, we fitted a cumulative normal distribution of anti-circumsporozoite antibody titre and protection. This model can approximate both an abrupt and a more gradual change in protection between the two groups.

The cumulative normal distribution that fit our data had an SD of 0.1 on a log scale (95% CI 0.05–0.3), indicating that 70% of the change in level of protection occurred between 30 EU/mL and 50 EU/mL (figure 5). In calculating the uncertainty around this bootstrap estimate, we noted that the fifth percentile indicates the most abrupt transition, with 70% of the change in level of protection occurring between 35 EU/mL and 45 EU/mL, and that the 95th percentile indicates a more gradual transition with 70% of the change occurring between 16 and 100 EU/mL (figure 5). Other non-linear fits with power functions and fractional polynomials were not significantly different from the linear model (data not shown).

Discussion

RTS,S/AS01E provides sustained protection from clinical malaria over a period of 15 months (range 12–18 months) in young children residing in malaria endemic areas. Estimates of efficacy over 8 months follow-up from both sites,⁶ 12 months from both sites, and 15 months from Kilifi alone have overlapping CIs, consistent with sustained vaccine efficacy for at least 15 months. Furthermore, we recorded no waning in efficacy during the trial. Follow-up of the Kilifi cohort is continuing—data obtained will estimate more precisely the duration of protection.

A limitation of our study is that we recruited healthy children only; further studies are needed to establish vaccine efficacy in, for example, children with HIV infection or those who are malnourished. Furthermore, phase 3 studies should include study sites at different transmission intensities to confirm how generalisable our results are.

Antibody titres at the time of serological testing (rather than peak titres during the trial) predicted protection, probably because the time that sporozoites spend in the peripheral tissues and circulation before they invade hepatocytes is insufficient to elicit an anamnestic response.

In a preliminary post-hoc analysis, although we recorded no linear fit between antibody titres and risk of clinical malaria during the trial, individuals with antibody titres in the lowest tertile at month 6.5 after dose three had reduced protection compared with those in the higher two tertiles. This finding suggests a non-linear relation between anti-circumsporozoite antibody titres and protection conferred by RTS,S/AS01E vaccination. Models that divided the antibody titres into two groups fit the data best when dichotomising at 40 EU/mL. However,

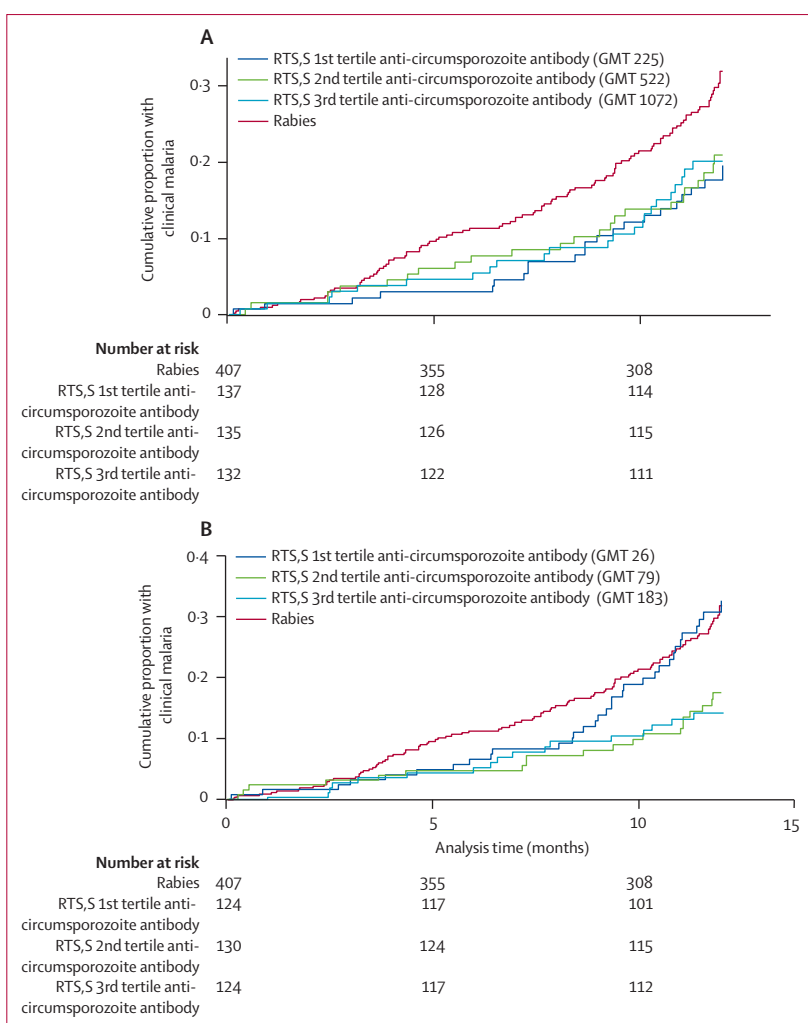


Figure 4: Kaplan-Meier curves showing the cumulative proportion of clinical falciparum malaria, by anti-circumsporozoite antibody tertiles

Clinical falciparum malaria defined as the presence of fever (temperature $\geq 37.5^{\circ}\text{C}$) and a *Plasmodium falciparum* density of 2500/ μL or more. Individuals who received RTS,S/AS01E, by tertile of anti-circumsporozoite antibody titres measured 1 month after dose three (A). Individuals who received RTS,S/AS01E, by tertile of anti-circumsporozoite antibody titres measured 6.5–10 months after dose one (B). GMT=geometric mean titre.

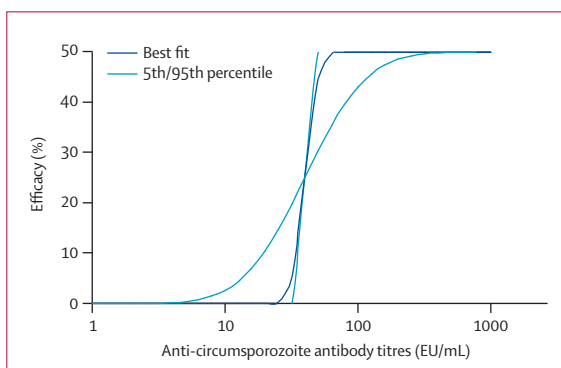


Figure 5: Anti-circumsporozoite antibody titres and efficacy

The modelled relation between anti-circumsporozoite antibody titres and efficacy is shown for a cumulative normal distribution, fit by maximising the log likelihood estimate. 5th and 95th percentiles calculated by bootstrap.

Panel: Research in context**Systematic review**

We searched PubMed with the terms ((“RTS,S-AS02D vaccine “[Substance Name] OR “RTS,S-AS01E vaccine “[Substance Name] OR “RTS,S-AS01B vaccine “[Substance Name] OR “RTS,S-AS02A vaccine “[Substance Name] OR “PfCSP DNA vaccine “[Substance Name])) AND “Malaria, falciparum/ prevention and control”[Mesh]. There are no studies describing the risk of clinical malaria over a similar length of follow-up after vaccination with RTS,S/AS01E. Previous studies have examined the relation between anti-circumsporozoite antibody concentrations and risk of *Plasmodium falciparum* infection or clinical malaria, but our study seems to be the first to provide a detailed analysis of concurrent antibody concentrations and to examine for non-linear effects.

Interpretation

RTS,S/AS01E confers 45–8% efficacy against clinical malaria during 15 months of follow-up in children residing in a malaria endemic area (95% CI 24.1–61.3). Anti-circumsporozoite antibody titres at the time of serological testing, rather than peak post-vaccination antibody titres predicted efficacy, and dichotomising the anti-circumsporozoite antibody titres at 40 EU/mL best predicted outcome.

the wide CIs recorded indicate that our model needs to be validated on other datasets.

Our findings need to be discussed in context with findings from previous studies (panel). Some studies have shown a linear relation between increasing anti-circumsporozoite antibody titres and protection. When the range of antibody titres studied includes 40 EU/mL, findings are consistent with our two-group model (or step function).^{13,14} However, two previous studies contradict our model. In Mozambican children, sustained protection against clinical malaria was seen until 18 months after vaccination despite mean anti-circumsporozoite antibody titres falling to 14 EU/mL. In sporozoite challenge studies, a linear association between anti-circumsporozoite antibody titres and protection against infection was seen over a range of titres above our dichotomisation point.⁹ Our model would have predicted very little protection at 18 months in the Mozambique study and no association between protection and antibody titres in the challenge studies. However, the model parameters we calculated have much uncertainty. For instance, the broadest step function, indicated by the 95th percentile for bootstrapped estimates, would predict low protection at 18 months in the Mozambique study and a linear fit over the range of the antibody titres seen in the challenge studies. The point of dichotomisation identified in this study might be specific to our data; a point substantially lower than 40 EU/mL would fit the Mozambique results despite an abrupt step function.

Cell-mediated immunity could be associated with protection.¹⁷ A partly effective pre-erythrocytic vaccine might either positively¹⁸ or negatively¹⁹ affect naturally acquired immunity to blood-stage parasites. Until further data are available, a single correlate to predict clinical outcomes should not be used. Furthermore, antibody titres could be a non-causal correlate with protection. For instance, sustained antibody titres might have a close relation with an additional mechanism, such as a cellular response.^{9,17} Alternatively, other properties of the antibody response might be more accurate correlates, such as antibody isotype, avidity, or functional properties, or their ability to bind to intact sporozoites.

The step function was a good statistical fit for our data, but is it biologically plausible? Increasing antibody titres could steadily inhibit more sporozoites until near to 100% vaccine efficacy is reached, rather than reach a plateau at 50% vaccine efficacy, as was seen in this trial. However, subpopulations of sporozoites could exist that reach the liver through different routes and after spending different lengths of time in the skin.^{20,21} A given anti-circumsporozoite antibody titre might inhibit only a subpopulation of sporozoites that take a particular route.²²

Vaccine development requires empirical evidence, which means that costly and time-consuming clinical studies are needed. Models that predict efficacy from immunological studies might greatly accelerate malaria vaccine development.

Contributors

All authors were involved in the design, implementation, and interpretation of study results. AO, JL, LvS, and PB were involved in all phases of the study. AL and JV led the clinical team at GlaxoSmithKline (GSK) Biologicals. AO, PB, and MML led the data analysis. AO, PN, NM, JL, LvS, SM, SG, OA, TL, JG, AM, and PB were responsible for field and hospital activities and safety surveillance. BS and TV were the programme managers at PATH MVI. TC and PV were coordinators at PATH MVI. KOA, EJ, and DL coordinated laboratory work. M-CD is the malaria vaccine project manager at GSK Biologicals. KM, ER, CD, ML, WRB, and NP contributed to the design of the study, implementation, and interpretation. JC heads malaria Vaccine Research and Development at GSK Biologicals. The writing of the paper was led by AO and PB with input from all other investigators.

Conflicts of interest

AL, ML, DL, JV, M-CD, EJ, WRB, and JC are employees of GSK Biologicals. AL, JV, JG, M-CD, WRB, and JC own shares in GSK. WRB and JC are listed as inventors of patented malaria vaccines. PV, TC, BS, and TV are employees of PATH MVI, which supports the development and testing of several malaria vaccines and is funded by the Bill & Melinda Gates Foundation. All other authors declare that they have no conflicts of interest.

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