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Final results from a phase 3, individually randomised, controlled trial of the RTS,S/AS01 malaria vaccine in African infants and children, including an evaluation of the efficacy of a booster dose.

The RTS,S Clinical Trials Partnership

Summary

Background: The efficacy and safety of the RTS,S/AS01 candidate malaria vaccine during 18 months of follow-up have been published previously. Here we report the final results from the same trial, including the efficacy of a booster dose.

Methods: A total of 8922 children and 6537 young infants, aged 5-17 months and 6-12 weeks respectively at first vaccination, were randomized 1:1:1 to receive either three doses of RTS,S/AS01 at month (M) 0, 1, 2 and a booster dose at M20 (R3R group); three doses of RTS,S/AS01 and a dose of comparator vaccine at M20 (R3C); or a comparator vaccine at M0, M1, M2 and M20 (C3C [control group]). Children and young infants were followed for a median of 48 or 38 months after dose-1 respectively. Cases of clinical and severe malaria were captured through passive case detection. Serious adverse events (SAE) were recorded.

Findings: Vaccine efficacy (VE) against all episodes of clinical malaria from M0 until the end of the study (intention-to-treat [ITT] population) in children was 36.3% (95% CI 31.8; 40.5) in the R3R group and 28.3% (95% CI 23.3; 32.9) in the R3C group and against severe malaria VE was 32.2% (95% CI 13.7; 46.9) and 1.1% (95% CI -23.0; 20.5) in the R3R and R3C groups respectively. VE against clinical malaria in young infants from M0 until the end of the study was 25.9% (95% CI 19.9; 31.5) and 18.3% (95% CI 11.7; 24.4) in the R3R and R3C groups respectively and against severe malaria VE was 17.3% (95% CI -9.4; 37.5) and 10.3% (95% CI -17.9; 31.8) in the R3R and R3C groups respectively. Efficacy waned over time in both age groups. In children, an average of 1774 and 1363 cases of clinical malaria were averted per 1000 children vaccinated in the R3R and R3C groups, respectively. In young infants, an average of 983 and 558 cases of clinical malaria were averted in the R3R and R3C groups, respectively. Impact was greater in areas of higher malaria transmission. The frequency of SAEs overall was balanced between groups. However, meningitis was reported as an SAE in 11, 10 and one child in the R3R, R3C and C3C groups respectively. The

incidence of generalized convulsive seizures within seven days of RTS,S/AS01 booster was 2.2 and 2.5/1000 doses administered in young infants and children respectively.

Interpretation: RTS,S/AS01 prevented a substantial number of cases of clinical malaria over a three to four year period in young infants and children when administered with or without a booster dose, especially in areas with higher malaria transmission. Efficacy was enhanced by the administration of a booster dose in both age categories.

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Introduction

Considerable progress has been made in malaria control during the past decade but the burden of malaria in Africa remains high.[1] A malaria vaccine could be an important complement to existing control measures, and could help reduce morbidity and mortality in children.

RTS,S/AS01 is a recombinant protein candidate malaria vaccine that targets the circumsporozoite (CS) protein of *Plasmodium falciparum*, expressed by the malaria parasite at the pre-erythrocytic stage, in which part of the CS sequence is co-expressed with fused and free hepatitis B surface antigen,[2, 3] and formulated with the AS01 adjuvant. Prior studies have established the ability of RTS,S/AS01 to provide protective immunity.[4-6]

This phase 3, double blind (observer-blind), individually randomised, controlled trial was conducted between 2009 and early 2014 at 11 centres in sub-Saharan Africa situated in areas with different intensities of malaria transmission (Figure Supplement [S] 1). Study results prior to the booster dose, including the co-primary end-points and safety and efficacy during 18 months of follow-up have been reported previously.[7-9] Protection against clinical and severe malaria was observed in both children and young infants during the first 12-months after vaccination but protection waned over time in both age categories.[9] Here we report the efficacy, immunogenicity, safety and impact of RTS,S/AS01 in children and young infants followed to the end of the trial, including findings in those who received a booster dose of vaccine.

Methods

The trial protocol was approved by the ethical review board at each study centre and partner institution and by the national regulatory authority in each country (Tables S1a and S1b) and the trial was undertaken in accordance with the provisions of the Good Clinical Practice Guidelines.[10]

Study design and participants

Trial methods have been reported previously,[7-9, 11] and are described in the supplementary materials. This individually randomised, controlled, double-blind (observer-blind), phase 3 trial was designed initially to evaluate vaccine efficacy (VE), safety, and immunogenicity during 32 months of follow-up but the protocol was amended prior to Month 32 and informed consent sought from each participant to extend the follow-up period until 31 December 2013 (median follow-up time 48 months for children and 38 months for young infants). Access to an insecticide treated bed net (ITN) was optimized for all screened children. Net usage and condition were determined during protocol-specified home visits.

Randomization and vaccination

From March 2009 until January 2011, 6,537 infants aged 6-12 weeks and 8,922 children aged 5-17 months were recruited and assigned randomly to one of three groups in a 1:1:1 ratio. One group received RTS,S/AS01 at month (M) 0, 1, and 2, followed by a booster dose at M20 (R3R); a second group received the RTS,S/AS01 primary vaccination series with meningococcal serogroup C conjugate vaccine (Menjugate™, Novartis) instead of a RTS,S/AS01 booster (R3C); the third group received only comparator vaccines, rabies vaccine (VeroRab™, Sanofi-Pasteur) for children or Menjugate™ (Novartis) for young infants (C3C [control group]) (Figure 1). Young infants received the study vaccine at the same time as Expanded Programme on Immunization (EPI) vaccines.

Procedures

Subjects did not receive malaria treatment prior to vaccination. The treatment of malaria cases during the course of the study was conducted in accordance with national guidelines. Malaria

was detected by passive surveillance. Clinical malaria was defined as an illness accompanied by an axillary temperature ≥37.5°C and *P. falciparum* asexual parasitaemia (>5000 parasites/mm^3) and severe malaria according to a predefined algorithm (Table S2). Case definitions for clinical and severe malaria are presented in tables 1, 2, 3 and S3. Severe malaria anaemia was defined as the presence of a haemoglobin concentration <5 g/dL and *P. falciparum* parasitaemia at any density, and malaria hospitalisation was defined as a hospital admission accompanied by *P. falciparum* parasitaemia at a density > 5000 parasites/mm^3. Information was collected on all unsolicited reports of adverse events (AEs) that occurred within 30 days after vaccination and on local and systemic reactogenicity within 7 days after vaccination among the first 200 participants enrolled at each centre, as described fully in the supplementary materials. Serious adverse events (SAEs) were identified during the entire study follow-up by surveillance at health facilities in the study area and through monthly home visits. Verbal autopsies, using standardized procedures, were conducted on deaths that occurred outside hospital. SAE were coded from clinician-assigned diagnoses according to the preferred terms of the *Medical Dictionary for Regulatory Activities*.[12] The case histories of all participants with reported meningitis or other CNS infections or inflammation were reviewed by two independent experts and designated as confirmed meningitis, not meningitis, or undetermined (supplementary methods). Anti-CS antibodies were measured by ELISA in the first 200 participants in each age category at each study site at enrolment, 1 and 18 months after the third dose of vaccine, 1 and 12 and 24 months after the booster dose and at the last study visit. The threshold for a positive titre was 0.5 EU/mL.[13] Other laboratory and radiologic procedures are described fully in the supplementary materials.

Statistical analysis

All results presented are for the intention-to-treat (ITT) population unless otherwise recorded as per-protocol (PP). The ITT population included all participants who received at least one dose of vaccine. The PP population included all participants who received three doses of vaccine according to protocol and contributed to the efficacy surveillance starting 14 days after the third dose. Efficacy against all episodes of malaria was analysed by negative binomial regression with follow-up time as offset, allowing for interdependence between episodes within the same subject. Overall estimates were adjusted for study site as a fixed effect, whereas site estimates were unadjusted for covariates. Inter-site variation was evaluated by site-interaction terms. VE over time was evaluated by calculating VE during consecutive time periods, M0-M20, M21-M32, and M33-study end (SE). The incremental efficacy of the RTS,S/AS01 booster dose was calculated for the time period after M20, when the booster dose was administered, and was calculated as 1 minus the incident rate ratio between the R3R and R3C groups. VE against severe end-points was estimated as a relative risk (RR) reduction with Fisher's exact *p*-values. The number of cases averted over time was calculated as the sum of three-monthly differences of the estimated number of cases between the control and the RTS,S/AS01 group (R3R+R3C up to the time of booster dose and R3R and R3C separately after the booster dose) and expressed per 1000 subjects vaccinated. Fourteen days following an episode were subtracted from the time at risk and no malaria events were counted during this period. Ninety-five percent confidence intervals were estimated by bootstrapping, using the 2.5 and 97.5 centiles of 1000 replicates obtained by sampling subjects, stratified by site.[14] The 32-month time period was selected to facilitate comparison of impact between young infants and children, who averaged different follow-up times, with relatively few young infants followed beyond M32. The primary case definition of clinical malaria was used for determination of VE, while a more sensitive secondary case

definition was used for the evaluation of impact on clinical malaria because, in clinical practice, sick children who present to a health facility with any level of malaria parasitaemia are likely to receive treatment for malaria. Data were censored at the end of the follow-up period, or at the date of emigration, withdrawal of consent, or death.

Role of the funding source

The study is sponsored by GSK Biologicals SA, the vaccine developer and manufacturer and funded by both GSK Biologicals SA and the PATH Malaria Vaccine Initiative (MVI). The study was designed by the Clinical Trial Partnership Committee (CTPC), consisting of representatives of all research sites, study sponsor and study funder.[11] GSK Biologicals SA coordinated the collection, analysis and interpretation of the data. The investigators from the RTS,S Clinical Trials Partnership obtained data and cared for the study participants. The CTPC had full access to the study data, made the decision to publish the manuscript in its current form and prepared the manuscript.

Results

Overall, 8922 children and 6537 young infants were enrolled and included in the ITT populations, while 6918 (78%) children and 5997 (92%) young infants were included in the PP populations (Figures 2a, 2b). Baseline characteristics were similar in the three study groups in each age category and between the ITT and PP populations but differed between sites (Figure S2). ITN usage was consistently high although it varied by study site (Figure S3). Malaria incidence in young infants in the C3C group during the first 12 months of follow-up ranged across sites from 0.03 to 4.27 episodes per infant per year (per-protocol, Table S4). Overall, 99% of children and young infants who presented to study clinics and received treatment for malaria were prescribed an artemisinin combination therapy (ACT)

(Figure S3). Mortality during the overall follow-up period (M0-SE) was relatively low in each study group, 1.8% (95% CI 1.5; 2.1) in children and 2.3% (95% CI 1.9; 2.7) in young infants (Tables S5 and S6).

Vaccine efficacy against all episodes of clinical malaria in children from M0 until SE in the R3R group was 36.3% (95% CI 31.8; 40.5) (ITT) and 39.0% (95% CI 34.3; 43.3) (PP) and in the R3C group efficacy was 28.3% (95% CI 23.3; 32.9) (ITT) and 26.2% (95% CI 20.8; 31.2) (PP) (Table 1, Tables S7, S8 and Figure S4). Efficacy was similar in children 5-11 months of age and 12-17 months of age (Table S9). Efficacy varied by site with or without booster vaccination although not reaching statistical significance at the 5% level (interaction test, p =0.09 and p = 0.11 respectively) (Figure 3). Efficacy waned over time and in the R3C group it was no longer detectable in the last study period [VE M33-SE = 2.9% (95% CI -6.4; 11.4)]. In contrast, VE persisted to SE in the R3R group [VE M33-SE = 12.3% (95% CI 3.6; 20.1)] (Table 1 and Figure S5). The added efficacy provided by the booster dose during the 12 months following booster vaccination was 25.6% (95% CI 18.2; 32.3) (Table 1 and Figure S6).

Vaccine efficacy against severe malaria in children from M0 until SE in the R3R group was 32.2% (95% CI 13.7; 46.9) (ITT) and 28.5% (95% CI 6.3; 45.7) (PP) but efficacy was not demonstrated in the R3C group [VE M0-SE = 1.1% (95% CI -23.0; 20.5) (ITT) and -5.8% (95% CI -35.0; 17.0) (PP)] (Tables 1, S7 and S8). Efficacy against severe malaria was present in children in the combined R3R+R3C group from M0 until M20 [VE M0-M20 = 33.9% (95% CI 15.3; 48.3)], but was not seen thereafter in either the R3R group [VE M21-SE = -4.0% (95% CI = -50.0; 27.8)] or in the R3C group [VE M21-SE = -41.0% (-98.5; -0.8)] in whom the incidence of severe malaria was significantly higher (p = 0.04) than in the C3C during this period (Table 1). Vaccine efficacy against severe malaria by site is shown in figure 3. The distribution of markers of severe malaria in the three study groups is shown in figure

S7; illnesses characterised by a low coma score were noted more frequently in children who had received RTS,S/AS01 but confidence intervals for comparisons between groups overlap. Ninety-six percent of children and 98% of infants admitted with severe malaria recovered without major lasting sequelae (Tables S10, S11).

Vaccine efficacy against incident severe malaria anaemia in children in the R3R and R3C groups from M0 to study end was 47.8% (95% CI 11.6; 69.9) and 22.7% (95% CI -23.8; 52.1) respectively, against malaria hospitalization 34.6% (95% CI 22.5; 44.9) and 17.5% (95% CI 3.3; 29.7), and against all cause hospitalization 16.5% (95% CI 7.2; 24.9) and 11.5% (95% CI 1.7; 20.3) respectively (Tables S12, S13). Fewer blood transfusions were given to children in the R3R or R3C groups compared with controls [VE against blood transfusion M0-SE = 28.5% (95% CI 3.5; 47.2) and VE M0-SE = 16.5% (95% CI -11.4; 37.5)] respectively (Table S13).

Statistically significant efficacy against prevalent parasitaemia was observed in the R3R group at the cross-sectional surveys at M32, M44 and SE, and in the R3C group at M32 but did not reach statistical significance at SE (Table S14). No significant VE was seen against incident bacteraemia, pneumonia, all-cause mortality or malaria mortality (Table S13) and there was no impact on indices of malnutrition with or without a booster dose (Table S15).

The number of cases averted from M0 until SE per 1000 children vaccinated in the R3R and R3C study groups respectively was 1774 (95% CI 1387; 2186) (range across sites: 205 to 6565) and 1363 (95% CI 995; 1797) (range: 215 to 4443) for clinical malaria (Figure 4 and Table S16); 19 and eight for severe malaria; 40 and 26 for malaria hospital admissions; 59 and 41 for all-cause hospital admissions; and 11 and nine for severe anaemia. Fifteen and 13 blood transfusions were averted per 1000 children vaccinated in the R3R and R3C groups respectively (Table 2). The impact of the vaccine against these end-points varied substantially

by study site; higher impact against clinical malaria was seen in areas of higher malaria incidence (Figure 4).

Vaccine efficacy against all episodes of clinical malaria in young infants from M0 until SE in the R3R group was 25.9% (95% CI 19.9; 31.5) (ITT) and 26.7% (95% CI 20.5; 32.4) (PP) and in the R3C group it was 18.3% (95% CI 11.7; 24.4) (ITT) and 18.2% (95% CI 11.4; 24.5) (PP) (Tables 3, S17 and S18, Figure S8). Estimates of vaccine efficacy across sites ranged from -14.6% to 50.4% in the R3R group and from -2.8% to 33.6% in the R3C group (Figure 5). Efficacy waned over time but was still present in the R3R group during the last study period (VE M33-SE = 10.5% [95% CI 0.2; 19.7]) but not in the R3C group (VE M33-SE = 4.4% [95% CI -6.7; 14.3]) (Table 3, Figure S9). The incremental efficacy against clinical malaria provided by the booster dose during the 12 months following booster vaccination was 22.3% (95% CI 14.0; 29.8) (Table 3, Figure S10).

Statistically significant VE against severe malaria throughout the study period was not seen in young infants in either the R3R or R3C group (VE M0-SE = 17.3% [95% CI -9.4; 37.5] and 10.3% [95% CI -17.9; 31.8], respectively) (Table 3). Vaccine efficacy against severe malaria anaemia among young infants in the R3R and R3C groups respectively was 31.5% (95% CI -18.5; 61.0) and 11.4% (95% CI -47.9; 47.2) (Table S19). Vaccine efficacy against malaria hospitalization among young infants in the R3R and R3C groups was 24.5% (95% CI 5.6; 39.7) and 11.1% (95% CI -10.1; 28.3) respectively (Table S19). No protection was demonstrated against all-cause hospitalisation, bacteraemia, pneumonia, all-cause mortality or malaria mortality. There was no VE against prevalent parasitaemia or indices of malnutrition with or without a booster (Tables S20, S21, S22).

The number of cases averted per 1000 young infants vaccinated from M0 until SE in the R3R and R3C study groups respectively was 983 (95% CI 592; 1337) (range across sites: -30 to 3406) and 558 (95% CI 158; 926) (range: -172 to 2178) for clinical malaria (Figure 6 and

Table S23), 12 and eight for severe malaria, 18 and 14 for malaria hospital admissions, and 36 and 24 for all-cause hospital admissions (Figure 6 and Table 2).

Anti-CS antibody responses are shown in Figure 7. One month after the booster dose with RTS,S/AS01, the geometric mean titres (GMTs) in children in the R3R groups was 318.2 EU/mL (95% CI 295.1; 343.0) compared with a titre of 34.2 EU/mL (95% CI 30.5; 38.3) in the R3C group (PP population) (Table S24). The comparable figures in young infants were 169.9 EU/mL (95% CI 153.8; 187.7) and 6.2 EU/mL (95% CI 5.4; 7.0) respectively (PP population) (Table S25). Antibody concentrations fell after the increase induced by the booster dose and 12 months later were 52.4 EU/mL (95% CI 47.8; 57.6) and 19.3 EU/mL (95% CI 17.2; 21.8) in children and 15.9 EU/mL (95% CI 13.8; 18.3) and 3.7 EU/mL (95% CI 3.3; 4.2) in young infants in the R3R and R3C groups respectively (Figure 7, Tables S24 and S25). Anti-CS antibodies were categorised by tertile. Infants who were RTS,S/AS01 recipients and whose antibody response was in the top tertile one month post primary vaccination series had a 36.9% (95% CI 17.3; 51.8; p=0.0009) reduction in risk of subsequent malaria episodes compared to those in the lowest tertile. No significant risk reduction was observed in children in the highest tertile compared to children in the lowest tertile (Figures S11 and S12).

The RTS,S/AS01 booster dose was more reactogenic than the comparator vaccine in both children and young infants, with a higher frequency of both systemic and local reactions within seven days of vaccination in the R3R group than in the R3C or C3C groups (Tables S26 and S27). However, grade 3 reactions were rare, except for grade 3 fevers (>39^{o} C) which occurred in 5.3% (95% CI 3.7; 7.3) of children and 1.5% (95% CI 0.7; 2.8) of infants following a booster dose of RTS,S/AS01 (Figures S13, S14 and Tables S26, S27). The incidence of generalised convulsive seizures within seven days of a booster dose was 2.5, 1.2

and 0.4 per 1000 doses in children and 2.2, zero and 0.5 per 1000 doses in infants in the R3R, R3C and C3C groups respectively (Table S28).

The incidence of SAEs overall and of unsolicited AEs within 30 days of booster vaccination was similar in all three study groups (Tables S5, S6 and S29 to S32). Meningitis was reported as an SAE in 22 children, 11, 10 and one in the R3R, R3C and C3C groups respectively. Five of these 22 cases were reported after booster vaccination, two in the R3R group, three in the R3C group and none in the C3C group. A bacterial aetiology was identified in ten cases (five meningococcal, three *Haemophilus influenzae*, one pneumococcal, and one tuberculosis), all in children in the R3R or R3C group. Meningitis was reported as an SAE in 18 young infants; no imbalance in cases of meningitis was seen in the younger age group (five cases in the R3R group, seven cases in the R3C group, and six cases in the C3C group). Meningitis cases were not temporally related to vaccination (Figure S15). Most study sites reported 1-3 cases of meningitis, but 15 cases were reported from Lilongwe, Malawi and five from Kombewa, Kenya. All cases of meningitis and other infection of the nervous system reported as an SAE were reviewed by two independent experts. In children, 33 episodes were reviewed by the experts who classified 12 as confirmed cases of meningitis (six in R3R, six in R3C), 11 as not a meningitis case (five in R3R, four in R3C and two in C3C) and 10 as undetermined (five in R3R, two in R3C and three in C3C). In infants, 22 episodes were reviewed by the experts who classified 11 as confirmed cases of meningitis (three in R3R, five in R3C, three in C3C), eight as not meningitis cases (two in R3R, two in R3C and four in C3C) and three as undetermined (two in R3R, one in R3C).

Discussion

We have reported previously findings in children who received a primary course of vaccination with the RTS,S/AS01 malaria vaccine at the ages of 6-12 weeks or 5-17 months

and who were followed for a period of 18 months.[7-9] We now report findings in these two age-categories followed for median periods of 38 and 48 months respectively since first vaccination, including findings in the half of the RTS,S/AS01 vaccinated children and young infants who received a booster dose of RTS,S/AS01 18 months after the primary course.

The decline in efficacy of RTS,S/AS01 against clinical and severe malaria with time since vaccination, reported previously,[9] continued during the extended follow-up period in both children and infants who did not receive a booster dose of vaccine. Nevertheless, ITT efficacies against clinical malaria during the full follow-up period in the absence of a booster dose were 28.3% in children and 18.3% in young infants. This resulted in an average reduction in cases of clinical malaria across the 11 study sites of 1363 (95% CI 995; 1797) per 1000 children vaccinated over a median follow-up period of 48 months and of 558 (95% CI 158; 926) per 1000 young infants vaccinated over a median follow-up period of 38 months. Protection against clinical malaria was prolonged in both children and young infants by a booster dose, increasing the average number of cases prevented across sites to 1774 (95% CI 1387; 2186) in children and 983 (95% CI 592; 1337) in young infants. The proportional increase in efficacy against clinical malaria associated with booster vaccination was similar in children and young infants but efficacy after the booster dose remained lower in those who received their primary vaccination when aged 6-12 weeks rather than at the age of 5-17 months. No significant efficacy against severe malaria was seen during the overall study period in young infants or children who did not receive a booster dose of vaccine. This contrasts with our earlier report of a statistically significant reduction in severe malaria in children in the older age category followed until the time of booster vaccination.[8, 9] This anomaly is explained by the higher risk of severe malaria from month 21 until the end of the trial in children who did not receive a booster dose of vaccine compared to children who received a comparator vaccine. This increased risk was seen predominantly in sites with a

higher level of malaria transmission. Why children who received the RTS,S/AS01 primary vaccination series, but who did not receive a booster dose were at increased risk of severe malaria during the latter part of the study is uncertain. This may have been a chance finding; the number of cases was low and it was not observed for cases of uncomplicated malaria. However, it is possible that vaccination, by providing protection against malaria infection, reduced the natural acquisition of immunity obtained through repeated infections making these children more susceptible when the vaccine effect waned. This possibility needs to be explored in further studies and on-going surveillance of study participants is taking place at three centres. The increased risk for severe malaria in the older age-category was reduced by the administration of a booster dose of RTS,S/AS01. This finding suggests that if a decision is made to implement RTS,S/AS01 in an age category similar to that of the older children recruited to this trial, then strong consideration should be given to inclusion of a booster dose, especially in higher transmission areas and the possible impact of administration of further booster doses will need to be explored.

Vaccination with RTS,S/AS01 significantly reduced overall hospital admissions, admissions due to malaria, severe anaemia and the need for blood transfusion in children, with these protective effects being more marked in those who received a booster dose. Much less impact against these end-points was seen in infants vaccinated at 6-12 weeks of age. No significant impact on overall mortality, malaria mortality, pneumonia or sepsis was observed in either age category. The latter is surprising as there is strong evidence that malaria is an important risk factor for invasive bacterial infections.[15] Failure to detect an impact of RTS,S/AS01 on these secondary outcomes, including mortality, may have been due in part to the high level of clinical care provided during the trial, including high coverage with ITNs and enhanced access to effective treatment of malaria and other conditions. The impact of

RTS,S/AS01 on these end-points might be higher in communities where access to a high level of clinical care is less readily accessible than was the case during the trial.

Administration of a booster dose of RTS,S/AS01 led to an increase in anti-CS GMT in both young infants and children, as noted previously in adults immunised with an earlier formulation of the vaccine (RTS,S/AS02),[16] but the post-booster anti-CS GMTs remained lower than post-primary levels and the booster effect was only transitory. Changes in anti-CS concentration over time paralleled changes in efficacy against clinical malaria. No previously unvaccinated children vaccinated with a single dose of RTS,S/AS01 at the same age as the children who received the booster dose were included in the trial, so it is not possible to conclude definitively that children who received the booster dose had acquired immunological memory. However, limited data from previous studies suggest that the antibody response to a booster dose in children who had been primed with RTS,S/AS01 was greater than that seen in subjects who had received just a single dose without priming, and that some immunological memory had been induced with the prime series of RTS,S/AS01 vaccination.[17] Further studies are needed to define the mechanism of memory induced with RTS,S/AS01 and whether there are ways in which this could be improved.

SAE were reported in approximately one quarter of children in the trial with a similar incidence in all study groups but only 0.3% were considered to be vaccine related. However, the significant imbalance in cases of meningitis in children vaccinated at the age of 5-17 months between the RTS,S/AS01 and control groups reported previously, remained. Five new cases of meningitis were recorded from month 21 until the end of the trial in children in the RTS,S/AS01 group but none in the control group; two of the five new cases occurred in children who had received the booster dose of RTS,S/AS01 and three in children who had received the control meningococcal serogroup C vaccine. The imbalance in cases of meningitis was not seen in young infants. This imbalance in cases of meningitis in children

could be a chance finding as comparisons were made across groups for many different diagnostic classifications of SAE, most of the cases were clustered in two sites, and there was no temporal relationship to vaccination. If children who received RTS,S/AS01 do have a true increased risk of meningitis, it is difficult to understand the mechanism that could have brought this about. If RTS,S/AS01 is licensed, post-registration studies will be performed to determine the significance of this finding. The incidence of fever in the week after vaccination was higher in both infants and children who received a booster dose of RTS,S/AS01 vaccine than in those who received the control vaccine, as noted during the primary series of vaccination, and a small number of these febrile reactions (2.5 per 1000 doses) were accompanied by generalized convulsive seizures.

Despite its large size and attention to detail, this trial still had some weaknesses. The PP population was high in young infants (92%) but lower in children (78%) due to loss of data from one centre following administration of vaccine affected by a temperature deviation. At one centre, Bagamoyo, there was a concern about the performance of two field workers assigned to perform monthly home visits, but further investigation found no evidence that this had led to under-reporting of SAEs. Differences in the impact of RTS,S/AS01 were found between study sites but because of the relative infrequency of some of the trial end-points, including severe malaria, site to site comparisons need to be approached with caution. The detailed study analyses conducted at several time points generated many hundreds of comparisons and created the opportunity for some unexpected associations to emerge by chance. Finally, the high standard of care provided to all trial participants may have limited the ability of the trial to detect an impact on mortality or other severe outcomes.

An application for a CHMP (Committee for Medicinal Products for Human Use) scientific opinion on RTS,S/AS01 through the European Medicines Agency (EMA) Article 58 procedure is currently under review. If a positive scientific opinion is obtained from the

CHMP and the vaccine is pre-qualified by WHO, malaria endemic countries will need to decide whether to license and deploy RTS,S/AS01 and, if so, what schedule to use. In anticipation of a positive opinion from the CHMP, WHO has established a Joint Technical Expert Group (JTEG) http://www.who.int/immunization/research/committees/jteg/en/ to monitor progress with the RTS,S/AS01 trials with the intention that this group will provide advice to a joint committee of WHO's Malaria Policy Advisory Committee (MPAC) and the Strategic Advisory Group of Experts (SAGE) committees which will formulate WHO's recommendations on the use of RTS,S/AS01. The results provided from this phase 3 trial should help these groups in making their decisions and, if RTS,S/AS01 is licensed in African countries, help national malaria control programmes in deciding how best to use this vaccine which, if deployed correctly, has the potential to prevent millions of cases of malaria.

Words: 4748

Panel: Research in context

Evidence before this study

We did a systematic literature search between December 2014 and February 2015 on randomized controlled trials of RTS, S malaria vaccine on PubMed, the Cochrane Library and other relevant data sources for the period 1984 to 31 January 2015. PubMed was searched using the MeSH terms: "RTS, S-AS01B vaccine"[All Fields] OR "RTS, S-AS01E vaccine"[All Fields] OR "RTS, S-AS02A vaccine"[All Fields] OR "RTS, S-AS02D vaccine"[All Fields] OR "RTS, S/AS01"[All Fields] OR "RTS, S/AS02"[All Fields] AND "clinical trial"[Publication Type] OR "clinical trials as topic"[MeSH Terms] OR "clinical trial"[All Fields] AND "humans"[MeSH Terms]. For the Cochrane Library and other data sources, the key search terms used were “RTS, S”, “Malaria Vaccines” AND “Clinical Trials”. The 60 papers identified included five which reported the results of randomized controlled trials with long term safety or efficacy follow-up or booster dose [18-22], two pooled analyses [23-24] and two systematic reviews [25-26].

Added value of this study

This study provided additional information on the safety and long term efficacy of RTS,S/AS01 in a large population of children across different malaria transmission settings. In addition, the study demonstrated how booster vaccination extended the period of protection provided by the vaccine.

Implications of all available evidence

The RTS, S malaria vaccine candidate has consistently shown protection against clinical malaria episodes in different age groups across different transmission settings. Vaccine efficacy has been shown with or without concurrent EPI vaccination. The vaccine has consistently shown a good safety profile although a meningitis safety signal reported among older children will require further follow-up. The results of the current demonstrate the

potential public health benefit of the RTS, S vaccine as an additional tool for malaria control whilst the next generations of malaria vaccines are being developed.

Disclaimer:

The opinions and assertions herein are the views of the author and not the Department of Defense or US Government.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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FIGURES

Figure 1 Study design

M = study month; SE = study end. The study was extended until end of December 2013. The total follow-up time of participants varied depending on their enrolment date. The median follow-up time post dose-1 in the 6-12 weeks was R3R=37·8 months; R3C=37·7 months; C3C=37·8 months (median overall: 38 months). The median follow-up time post dose-1 in the 5-17 months was R3R=48·1 months; R3C=48·1 months; C3C=48·4 months (median overall: 48 months).

Figure 2A. Participants' flow in the study. Consort for children enrolled in the 5-17 months age category.

CW = consent withdrawal.

FU = follow-up.

ITT = intention-to-treat.

PP = per-protocol.

a. For 70 children in the 5-17 months age category, the screening data had not been reported before the database freeze of the previous analyses and these subjects were not included in the consort charts published previously.

b. During monitoring, it was found that one subject belonging to the 5-17 months age category was enrolled twice at two different clinics under two different subject numbers. This subject was excluded from the per-protocol analyses. Due to the removal of one subject number from the database, the total number of subjects enrolled into the study changed from 15460 subjects (8923 in 5-17 months) as reported in previous analyses to 15459 subjects (8922 in 5-17 months) in the final analyses reported here.

Figure 2B. Participants' flow in the study. Consort for infants enrolled in the 6-12 weeks age category.

CW = consent withdrawal.

FU = follow-up.

ITT = intention-to-treat.

PP = per-protocol.

a. For 118 infants in the 6-12 weeks age category, the screening data had not been reported before the database freeze of the previous analyses and these subjects were not included in the consort charts published previously.

b. For some subjects, consent to the extension occurred before Visit 34. One subject consented to the extension but died before performing Visit 34 and appears in the reason for not completing Visit 34 as "died". This subject is considered as enrolled into the extension and the reason for not performing the Visit 38 is recorded as 'died".

Figure 3. Vaccine efficacy against clinical and severe malaria by study site in the 5-17 months age category, ordered by increasing malaria incidence at each site (intention-to-treat population).

A. Vaccine efficacy against all episodes of clinical malaria (primary case definition) in the R3C group (M0-SE)

B. Vaccine efficacy against all episodes of clinical malaria (primary case definition) in the R3R group (M0-SE)

C. Vaccine efficacy against severe malaria (primary case definition) in the R3C group (M0-SE)

D. Vaccine efficacy against severe malaria (primary case definition) in the R3R group (M0-SE)

The size of each blue square reflects the relative number of subjects enrolled at each study site; the horizontal bars show the lower limits and upper limits of the 95% confidence intervals. Study sites are ordered from lowest (Kilifi) to highest (Siaya) incidence of clinical malaria, defined as a measured or reported fever within previous 24h and parasite density >0 parasites per cubic millimetre (i.e. clinical malaria secondary case definition), measured in control infants 6-12 weeks of age at enrolment during 12 months of follow-up.

R3R = RTS,S/AS01 primary schedule with booster.

R3C = RTS,S/AS01 primary schedule without booster.

M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase).

VE = vaccine efficacy against all episodes of clinical malaria or severe malaria meeting the primary case definition.

LL = lower limit of the 95% confidence interval.

UL = upper limit of the 95% confidence interval.

Figure 4. Cases averted of clinical and severe malaria at each site during 48 months of follow-up in the 5-17 months age category, ordered by increasing malaria incidence at each study site (intention-to-treat population).

A. Cases averted of clinical malaria (secondary case definition) (M0-SE)

B. Cases averted of severe malaria (secondary case definition) (M0-SE)

R3R = RTS,S/AS01 primary schedule with booster.

R3C = RTS,S/AS01 primary schedule without booster.

M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase).

Clinical malaria secondary case definition = Illness in a child brought to a study facility with a measured temperature of ≥37.5°C or reported fever within the last 24 hours and *P. falciparum* asexual parasitaemia at a density of > 0 parasites per cubic millimetre. This definition was

used for this analysis as, during routine clinical practice, these children would normally receive a full course of anti-malarial treatment.

Severe malaria secondary case definition = *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre with one or more markers of disease severity, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of ≤ 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated lactate level, or haemoglobin level of < 5 g per decilitre. Coexisting illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.

Figure 5. Vaccine efficacy against clinical and severe malaria by study site in the 6-12 weeks age category, ordered by increasing malaria incidence at each study site (intention-to-treat population).

A. Vaccine efficacy against all episodes of clinical malaria (primary case definition) in the R3C group (M0-SE)

B. Vaccine efficacy against all episodes of clinical malaria (primary case definition) in the R3R group (M0-SE)

C. Vaccine efficacy against severe malaria (primary case definition) in the R3C group (M0-SE)

D. Vaccine efficacy against severe malaria (primary case definition) in the R3R group (M0-SE)

The size of each blue square reflects the relative number of subjects enrolled at each study site; the horizontal bars show the lower limits and upper limits of the 95% confidence

intervals. Study sites are ordered from lowest (Kilifi) to highest (Siaya) incidence of clinical malaria, defined as a measured or reported fever within previous 24h and parasite density >0 parasites per cubic millimetre (i.e. clinical malaria secondary case definition), measured in control infants 6-12 weeks of age at enrolment during 12 months of follow-up.

R3R = RTS,S/AS01 primary schedule with booster.

R3C = RTS,S/AS01 primary schedule without booster.

M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase).

VE = vaccine efficacy against all episodes of clinical malaria or severe malaria meeting the primary case definition.

LL = lower limit of the 95% confidence interval.

UL = upper limit of the 95% confidence interval.

Figure 6. Cases averted of clinical and severe malaria in each site during 38 months of follow-up in the 6-12 weeks age category, ordered by increasing malaria incidence at each study site (intention-to-treat population).

A. Cases averted of clinical malaria (secondary case definition) (M0-SE)

B. Cases averted of severe malaria (secondary case definition) (M0-SE)

R3R = RTS,S/AS01 primary schedule with booster.

R3C = RTS,S/AS01 primary schedule without booster.

M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase).

Clinical malaria secondary case definition = Illness in a child brought to a study facility with a measured temperature of ≥37.5°C or reported fever within the last 24 hours and *P. falciparum* asexual parasitaemia at a density of > 0 parasites per cubic millimetre. This definition was used for this analysis as, during routine clinical practice, these children would normally receive a full course of anti-malarial treatment.

Severe malaria secondary case definition = *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre with one or more markers of disease severity, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of ≤ 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated lactate level, or haemoglobin level of < 5 g per decilitre. Coexisting illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.

Figure 7. Anti-CS geometric mean titres in each age category (per-protocol population for immunogenicity)

R3R+R3C = RTS,S/AS01 primary schedule (combined R3R + R3C groups analysed over the period before the administration of the booster dose at Month 20).

R3R = RTS,S/AS01 primary schedule with booster.

R3C = RTS,S/AS01 primary schedule without booster.

Anti-CS = anti-circumsporozoite protein antibodies.

GMT = geometric mean antibody titre calculated on all subjects.

EU/mL = ELISA unit per millilitre.

TABLES

Table 1. Overall vaccine efficacy against clinical and severe malaria in the 5-17 months age category (intention-to-treat population).

Efficacy against clinical malaria (primary case definition) of a primary schedule without booster (R3C)												
		R3C				C3C				Point estimate of VE unadjusted for covariates			
Intention-to-treat population		**N**	**n**	**T (year)**	**n/T**	**N**	**n**	**T (year)**	**n/T**	**(%)**	**95% CI**		**p-value**
Clinical malaria	M0-SE	2972	7396	10037·3	0·74	2974	9585	9994·9	0·96	28·3	23·3	32·9	<0·0001
	M0-M32	2972	4711	7180·0	0·66	2974	6768	7088·5	0·95	35·2	30·5	39·5	<0·0001
	M0-M20*	5949	5106	9059·1	0·56	2974	4305	4484·4	0·96	45·1	41·4	48·7	<0·0001
	M21-M32	2717	2076	2621·7	0·79	2700	2442	2609·9	0·94	16·1	8·5	23·0	<0·0001
	M33-SE	2267	2685	2861·6	0·94	2309	2817	2912·0	0·97	2·9	-6·4	11·4	0·5275
	M21-SE	2719	4761	5479·1	0·87	2701	5259	5516·3	0·95	11·4	4·4	18·0	0·0020
Efficacy against severe malaria (primary case definition) of a primary schedule without booster (R3C)												
		R3C				C3C				Point estimate of VE unadjusted for covariates			
Intention-to-treat population		**N**	**n**	**Proportion affected**		**N**	**n**	**Proportion affected**		**(%)**	**95% CI**		**p-value**
Severe malaria	M0-SE	2972	169	0·06		2974	171	0·06		1·1	-23·0	20·5	0·9555
	M0-M32	2972	145	0·05		2974	152	0·05		4·5	-20·6	24·5	0·7210
	M0-M20*	5949	156	0·03		2974	118	0·04		33·9	15·3	48·3	0·0007
	M21-M32	2717	61	0·02		2701	42	0·02		-44·4	-119·0	4·1	0·0730
	M33-SE	2267	31	0·01		2309	20	0·01		-57·9	-192·0	12·8	0·1216
	M21-SE	2719	88	0·03		2702	62	0·02		-41·0	-98·5	-0·8	0·0383
Efficacy against clinical malaria (primary case definition) of a primary schedule with booster (R3R)												
		R3R				C3C				Point estimate of VE unadjusted for covariates			
Intention-to-treat population		**N**	**n**	**T (year)**	**n/T**	**N**	**n**	**T (year)**	**n/T**	**(%)**	**95% CI**		**p-value**
Clinical malaria	M0-SE	2976	6616	9957·6	0·66	2974	9585	9994·9	0·96	36·3	31·8	40·5	<0·0001
	M0-M32	2976	4078	7099·7	0·57	2974	6768	7088·5	0·95	43·9	39·7	47·8	<0.0001
	M0-M20*	5949	5106	9059·1	0·56	2974	4305	4484·4	0·96	45·1	41·4	48·7	<0·0001
	M21-M32	2679	1592	2601·0	0·61	2700	2442	2609·9	0·94	37·4	31·4	42·8	<0·0001
	M33-SE	2236	2539	2862·2	0·89	2309	2817	2912·0	0·97	12·3	3·6	20·1	0·0062
	M21-SE	2681	4130	5458·9	0·76	2701	5259	5516·3	0·95	25·6	19·4	31·3	<0·0001
Efficacy against severe malaria (primary case definition) of a primary schedule with booster (R3R)												
		R3R				C3C				Point estimate of VE unadjusted for covariates			
Intention-to-treat population		**N**	**n**	**Proportion affected**		**N**	**n**	**Proportion affected**		**(%)**	**95% CI**		**p-value**
Severe malaria	M0-SE	2976	116	0·04		2974	171	0·06		32·2	13·7	46·9	0·0009
	M0-M32	2976	99	0·03		2974	152	0·05		34·9	15·6	50·0	0·0006
	M0-M20*	5949	156	0·03		2974	118	0·04		33·9	15·3	48·3	0·0007

	M21-M32	2679	43	0·02		2701	42	0·02		-3·2	-61·8	34·1	0·9132
	M33-SE	2236	23	0·01		2309	20	0·01		-18·8	-128·0	37·6	0·6466
	M21-SE	2681	64	0·02		2702	62	0·02		-4·0	-50·0	27·8	0·8572
Incremental efficacy against clinical malaria (Primary case definition) of a booster dose													
		R3R				**R3C**				**Point estimate of VE unadjusted for covariates**			
Intention-to-treat population		**N**	**n**	**T (year)**	**n/T**	**N**	**n**	**T (year)**	**n/T**	**(%)**	**95% CI**		**p-value**
Clinical malaria	M21-SE	2681	4130	5458·9	0·76	2719	4761	5479·1	0·87	16·2	9·1	22·7	<0·0001
	M21-M32	2679	1592	2601·0	0·61	2717	2076	2621·7	0·79	25·6	18·2	32·3	<0·0001

*Data from previous analysis (comparing R3R+R3C versus C3C).

R3R = RTS,S/AS01 primary schedule with booster.
R3C = RTS,S/AS01 primary schedule without booster.
C3C = Control group.
N = number of subjects.
n (clinical malaria) = number of episodes meeting the case definition.
n (severe malaria) = number of subjects reporting at least one event in each group.
T = person years at risk.
n/T = incidence rate.
Proportion affected = proportion of subjects reporting at least one event.
CI = confidence interval.
M = study month.
VE = vaccine efficacy (negative binomial model for clinical malaria; 1-relative risk for severe malaria).
M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase).
M0-M32 = follow-up from day of dose-1 (Month 0) to 32 months post dose-1 (Month 32).
M0-M20 = follow-up from day of dose-1 (Month 0) to 20 months post dose-1 (Month 20).
M21-M32 = follow-up from day of booster dose to 32 months post dose-1 (Month 32).
M33-SE = follow-up from start of extension phase to study end (end of extension phase).
M21-SE = follow-up from day of booster dose to study end (end of extension phase).
SE = study end (the median follow-up in the 5-17 months age category was 48 months post dose-1).
Clinical malaria primary case definition = illness in a child brought to a study facility with a measured temperature of ≥ 37.5°C and *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre or a case of malaria meeting the primary case definition of severe malaria.
Severe malaria primary case definition = *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre with one or more markers of disease severity and without diagnosis of a coexisting illness. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of ≤ 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated lactate level, or haemoglobin level of < 5 g per decilitre. Coexisting illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.
For clinical malaria: p-value from negative binomial regression.

For severe malaria: p-value from two-sided Fisher exact test.

Table 2. Number of cases averted in children in each age category immunized with a primary series of three doses of RTS,S/AS01 with or without a booster dose 18 months later (intention-to-treat population).

	Follow-up period	Number of cases averted per 1000 vaccinees in the 5-17 months age category		Number of cases averted per 1000 vaccinees in the 6-12 weeks age category	
		Schedule without a booster (R3C)	Schedule with a booster (R3R)	Schedule without a booster (R3C)	Schedule with a booster (R3R)
		n (LL ; UL)	n (LL ; UL)	n (LL ; UL)	n (LL ; UL)
Clinical malaria (secondary case definition)	M0-M20*	963 (807 ; 1133)		518 (341 ; 687)	
	M0-M32	1221 (973 ; 1483)	1475 (1234 ; 1733)	526 (200 ; 819)	873 (573 ; 1158)
	M0-SE	1363 (995 ; 1797)	1774 (1387 ; 2186)	558 (158 ; 926)	983 (592 ; 1337)
Malaria hospitalization	M0-M20*	42 (28 ; 59)		8 (-9 ; 25)	
	M0-M32	32 (13 ; 53)	44 (26 ; 64)	5 (-17 ; 27)	14 (-10 ; 35)
	M0-SE	26 (4 ; 51)	40 (19 ; 64)	14 (-13 ; 39)	18 (-8 ; 42)
Severe malaria (secondary case definition)	M0-M20*	19 (8 ; 32)		5 (-8 ; 18)	
	M0-M32	12 (-2 ; 27)	20 (7 ; 34)	5 (-13 ; 24)	9 (-8 ; 28)
	M0-SE	8 (-9 ; 26)	19 (4 ; 35)	8 (-13 ; 28)	12 (-6 ; 32)
Fatal malaria (ICD10 code)	M0-M20*	0 (-2 ; 3)		-1 (-3 ; 2)	
	M0-M32	-1 (-4 ; 3)	1 (-2 ; 4)	-1 (-5 ; 2)	-2 (-5 ; 2)
	M0-SE	-2 (-7 ; 2)	1 (-3 ; 5)	-3 (-7 ; 1)	-2 (-6 ; 2)
All cause hospitalization	M0-M20*	57 (29 ; 88)		22 (-16 ; 60)	
	M0-M32	53 (16 ; 92)	64 (29 ; 103)	15 (-32 ; 70)	38 (-9 ; 89)
	M0-SE	41 (0 ; 84)	59 (18 ; 103)	24 (-27 ; 82)	36 (-17 ; 90)
All-cause mortality	M0-M20*	-1 (-7 ; 4)		-5 (-12 ; 3)	
	M0-M32	-3 (-10 ; 3)	0 (-7 ; 6)	-5 (-13 ; 3)	-6 (-14 ; 3)
	M0-SE	-5 (-12 ; 3)	-1 (-9 ; 6)	-5 (-14 ; 4)	-6 (-15 ; 3)
Incident severe anaemia (case definition 3)	M0-M20*	8 (1 ; 15)		0 (-8 ; 11)	
	M0-M32	9 (0 ; 19)	10 (1 ; 20)	0 (-13 ; 15)	3 (-9 ; 16)
	M0-SE	9 (-3 ; 21)	11 (1 ; 24)	-1 (-16 ; 15)	3 (-11 ; 17)
Blood transfusion	M0-M20*	13 (4 ; 21)		1 (-10 ; 13)	
	M0-M32	13 (1 ; 24)	15 (3 ; 27)	2 (-15 ; 20)	5 (-9 ; 23)
	M0-SE	13 (-1 ; 28)	15 (1 ; 31)	1 (-18 ; 19)	4 (-12 ; 23)

Clinical malaria secondary case definition = illness in a child brought to a study facility with a measured temperature of ≥37.5°C or reported fever within the last 24 hours and *P. falciparum* asexual parasitaemia at a density of > 0 parasites per cubic millimetre. This definition was used for this analysis as, during routine clinical practice, these children would normally receive a full course of anti-malarial treatment.

Malaria hospitalization case definition 1 = a medical hospitalization with confirmed *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre. Severe malaria secondary case definition = *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre with one or more markers of disease severity, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of ≤ 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated

lactate level, or haemoglobin level of < 5 g per decilitre. Coexisting illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.
Fatal malaria (ICD10 code) = a fatal case associated with International Classification Disease (ICD10) code B50, B53, B54.
All-cause hospitalization primary case definition = a medical hospitalization of any cause, excluding planned admissions for medical investigation/care or elective surgery and trauma.
All-cause mortality case definition 1 = a fatality of any cause, including mortality in the community and in hospital.
Incident severe anaemia case definition 3 = a documented haemoglobin < 5.0 g per decilitre identified at clinical presentation to morbidity surveillance system.
Blood transfusion = a child with inpatient admission with documented blood transfusion.
n = number of cases averted per 1000 vaccinees.
CI = confidence interval.
LL = lower limit.
UL = upper limit.
M = study month.
M0-M20 = follow-up from day of dose-1 (Month 0) to 20 months post dose-1 (Month 20).
M0-M32 = follow-up from day of dose-1 (Month 0) to 32 months post dose-1 (Month 32).
M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase). For the 5-17 months age category SE= up to 48 months post dose-1, for the 6-12 weeks, SE= up to 39 months post dose-1.
* For M0-M20, the schedule without a booster (R3C) and the schedule with a booster (R3R) were pooled (R3R+R3C) to calculate the number of cases averted.

Table 3. Overall vaccine efficacy against clinical and severe malaria in the 6-12 weeks age category (intention to-treat population).

Efficacy against clinical malaria (primary case definition) of a primary schedule without booster (R3C)													
		R3C				**C3C**				**Point estimate of VE unadjusted for covariates**			
Intention-to-treat population		**N**	**n**	**T (year)**	**n/T**	**N**	**n**	**T (year)**	**n/T**	**(%)**	**95% CI**		**p-value**
Clinical malaria	M0-SE	2178	5444	6174·3	0·88	2179	6170	6147·3	1.00	18·3	11·7	24·4	<0·0001
	M0-M32	2178	4174	5190·7	0·8	2179	4916	5162·4	0·95	20·3	13·6	26·5	<0·0001
	M0-M20*	4358	4252	6583·6	0·65	2179	2751	3273·6	0·84	27·0	21·1	32·5	<0·0001
	M21-M32	1995	2079	1893·0	1·10	1976	2156	1889·3	1·14	7·6	-1·4	15·9	0·0958
	M33-SE	1658	1271	984·0	1·29	1657	1254	986·1	1·27	4·4	-6·7	14·3	0·4243
	M21-SE	1996	3349	2876·7	1·16	1976	3410	2874·2	1·19	7·6	-0·8	15·3	0·0758
Efficacy against severe malaria (primary case definition) of a primary schedule without booster (R3C)													
		R3C				**C3C**				**Point estimate of VE unadjusted for covariates**			
Intention-to-treat population		**N**	**n**	**Proportion affected**		**N**	**n**	**Proportion affected**		**(%)**	**95% CI**		**p-value**
Severe malaria	M0-SE	2178	104	0·05		2179	116	0·05		10·3	-17·9	31·8	0·4467
	M0-M32	2178	93	0·04		2179	101	0·05		7·9	-23·3	31·2	0·6073
	M0-M20*	4358	121	0·03		2179	66	0·03		8·3	-25·7	32·6	0·5819
	M21-M32	1995	40	0·02		1976	43	0·02		7·9	-45·1	41·6	0·7401
	M33-SE	1658	14	0·01		1657	16	0·01		12·6	-91·2	60·5	0·7188
	M21-SE	1996	52	0·03		1976	58	0·03		11·2	-31·3	40·2	0·5623
Efficacy against clinical malaria (primary case definition) of a primary schedule with booster (R3R)													
		R3R				**C3C**				**Point estimate of VE unadjusted for covariates**			
Intention-to-treat population		**N**	**n**	**T (year)**	**n/T**	**N**	**n**	**T (year)**	**n/T**	**(%)**	**95% CI**		**p-value**
Clinical malaria	M0-SE	2180	4993	6156·4	0·81	2179	6170	6147·3	1.00	25·9	19·9	31·5	<0·0001
	M0-M32	2180	3842	5173·3	0·74	2179	4916	5162·4	0·95	27·8	21·7	33·4	<0·0001
	M0-M20*	4358	4252	6583·6	0·65	2179	2751	3273·6	0·84	27·0	21·1	32·5	<0·0001
	M21-M32	1966	1671	1888·4	0·88	1976	2156	1889·3	1·14	28·1	20·6	34·8	<0·0001
	M33-SE	1654	1154	984·9	1·17	1657	1254	986·1	1·27	10·5	0·2	19·7	0·0457
	M21-SE	1966	2822	2871·5	0·98	1976	3410	2874·2	1·19	23·5	16·4	30·1	<0·0001
Efficacy against severe malaria (primary case definition) of a primary schedule with booster (R3R)													
		R3R				**C3C**				**Point estimate of VE unadjusted for covariates**			
Intention-to-treat population		**N**	**n**	**Proportion affected**		**N**	**n**	**Proportion affected**		**(%)**	**95% CI**		**p-value**
Severe malaria	M0-SE	2180	96	0·04		2179	116	0·05		17·3	-9·4	37·5	0·1598
	M0-M32	2180	89	0·04		2179	101	0·05		11·9	-18·3	34·5	0·3746
	M0-M20*	4358	121	0·03		2179	66	0·03		8·3	-25·7	32·6	0·5819
	M21-M32	1966	29	0·01		1976	43	0·02		32·2	-11·1	59·2	0·1216

	M33-SE	1654	12	0·01		1657	16	0·01		24·9	-69·3	67·6	0·5699
	M21-SE	1966	39	0·02		1976	58	0·03		32·4	-3·2	56·2	0·0638
Incremental efficacy against clinical malaria (Primary case definition) of a booster dose													
		R3R				**R3C**				**Point estimate of VE unadjusted for covariates**			
Intention-to-treat population		**N**	**n**	**T (year)**	**n/T**	**N**	**n**	**T (year)**	**n/T**	**(%)**	**95% CI**		**p-value**
Clinical malaria	M21-SE	1966	2822	2871·5	0·98	1996	3349	2876·7	1·16	17·5	9·5	24·8	<0·0001
	M21-M32	1966	1671	1888·4	0·88	1995	2079	1893·0	1·10	22·3	14·0	29·8	<0·0001

*Data from previous analysis (comparing R3R+R3C versus C3C).

R3R = RTS,S/AS01 primary schedule with booster.
R3C = RTS,S/AS01 primary schedule without booster.
C3C = Control group.
N = number of subjects.
n (clinical malaria) = number of episodes meeting the case definition.
n (severe malaria) = number of subjects reporting at least one event in each group.
T = person years at risk.
n/T = incidence rate.
Proportion affected = proportion of subjects reporting at least one event.
CI = confidence interval.
M = study month.
VE = vaccine efficacy (negative binomial model for clinical malaria; 1-relative risk for severe malaria).
M0-SE = follow-up from day of dose-1 (Month 0) to study end (end of extension phase).
M0-M32 = follow-up from day of dose-1 (Month 0) to 32 months post dose-1 (Month 32).
M0-M20 = follow-up from day of dose-1 (Month 0) to 20 months post dose-1 (Month 20).
M21-M32 = follow-up from day of booster dose to 32 months post dose-1 (Month 32).
M33-SE = follow-up from start of extension phase to study end (end of extension phase).
M21-SE = follow-up from day of booster dose to study end (end of extension phase).
SE = study end (the median follow-up in the 6-12 weeks was 38 months post dose-1).
Clinical malaria primary case definition = illness in a child brought to a study facility with a measured temperature of ≥ 37.5°C and *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre or a case of malaria meeting the primary case definition of severe malaria.
Severe malaria primary case definition = *P. falciparum* asexual parasitaemia at a density of > 5000 parasites per cubic millimetre with one or more markers of disease severity and without diagnosis of a coexisting illness. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of ≤ 2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia, acidosis, elevated lactate level, or haemoglobin level of < 5 g per decilitre. Coexisting illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.
For clinical malaria: p-value from negative binomial regression.
For severe malaria: p-value from two-sided Fisher exact test.