

Long-term incidence of severe malaria following RTS,S/AS01 vaccination in children and infants in Africa: an open-label 3-year extension study of a phase 3 randomised controlled trial



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Summary

Background Results from a previous phase 3 study showed efficacy of the RTS,S/AS01 vaccine against severe and clinical malaria in children (in 11 sites in Africa) during a 3–4-year follow-up. We aimed to investigate malaria incidence up to 7 years postvaccination in three of the sites of the initial study.

Methods In the initial phase 3 study, infants aged 6–12 weeks and children aged 5–17 months were randomly assigned (1:1:1) to receive four RTS,S/AS01 doses (four-dose group), three RTS,S/AS01 doses and a comparator dose (three-dose group), or four comparator doses (control group). In this open-label extension study in Korogwe (Tanzania), Kombewa (Kenya), and Nanoro (Burkina Faso), we assessed severe malaria incidences as the primary outcome for 3 additional years (January, 2014, to December, 2016), up to 6 years (younger children) or 7 years (older children) postprimary vaccination in the modified intention-to-treat population (ie, participants who received at least one dose of the study vaccine). As secondary outcomes, we evaluated clinical malaria incidences and serious adverse events. This trial is registered with ClinicalTrials.gov, number NCT02207816.

Findings We enrolled 1739 older children (aged 5–7 years) and 1345 younger children (aged 3–5 years). During the 3-year extension, 66 severe malaria cases were reported, resulting in severe malaria incidence of 0·004 cases per person-years at risk (PPY; 95% CI 0–0·033) in the four-dose group, 0·007 PPY (0·001–0·052) in the three-dose group, and 0·009 PPY (0·001–0·066) in the control group in the older children category and a vaccine efficacy against severe malaria that did not contribute significantly to the overall efficacy (four-dose group 53·7% [95% CI –13·7 to 81·1], $p=0\cdot093$; three-dose group 23·3% [–67·1 to 64·8], $p=0\cdot50$). In younger children, severe malaria incidences were 0·007 PPY (0·001–0·058) in the four-dose group, 0·007 PPY (0·001–0·054) in the three-dose group, and 0·011 PPY (0·001–0·083) in the control group. Vaccine efficacy against severe malaria also did not contribute significantly to the overall efficacy (four-dose group 32·1% [–53·1 to 69·9], $p=0\cdot35$; three-dose group 37·6% [–44·4 to 73·0], $p=0\cdot27$). Malaria transmission was still occurring as evidenced by an incidence of clinical malaria ranging from 0·165 PPY to 3·124 PPY across all study groups and sites. In older children, clinical malaria incidence was 1·079 PPY (95% CI 0·152–7·662) in the four-dose group, 1·108 PPY (0·156–7·868) in the three-dose group, and 1·016 PPY (0·14–7·213) in the control group. In younger children, malaria incidence was 1·632 PPY (0·23–11·59), 1·563 PPY (0·22–11·104), and 1·686 PPY (0·237–11·974), respectively. In the older age category in Nanoro, clinical malaria incidence was higher in the four-dose (2·444 PPY; $p=0\cdot011$) and three-dose (2·411 PPY; $p=0\cdot034$) groups compared with the control group (1·998 PPY). Three cerebral malaria episodes and five meningitis cases, but no vaccine-related severe adverse events, were reported.

Interpretation Overall, severe malaria incidence was low in all groups, with no evidence of rebound in RTS,S/AS01 recipients, despite an increased incidence of clinical malaria in older children who received RTS,S/AS01 compared with the comparator group in Nanoro. No safety signal was identified.

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Introduction

According to 2017 data from WHO, 20 million fewer cases of malaria occurred in 2017 than in 2010, but no substantial progress in reducing global malaria cases has been made between 2015 and 2017.¹ Most malaria

cases reported in 2017 were in the WHO African region (200 million cases [92%]), and more than half of malaria-related deaths occurred in children younger than 5 years, which is one of the most vulnerable groups.^{1,2}

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Research in context

Evidence before this study

Results from a phase 3 study have shown efficacy of the RTS,S/AS01 vaccine against a range of malaria endpoints over an average follow-up of 38 months (younger age category [6–12 weeks old]) to 48 months (older age category [5–17 months old]). However, vaccine efficacy waned over time and a fourth dose of vaccine was shown to extend the period of protection provided by vaccination. Interventions, including vaccination, aiming at reducing malaria morbidity might lead to a delayed acquisition of natural immunity and, ultimately, to an increased risk of malaria compared with controls not receiving the intervention. This effect is known as a rebound effect and has been documented for several malaria interventions. A previous extension of a phase 2 trial in Kenya showed such a rebound effect of uncomplicated malaria 5–6 years after receiving three doses of RTS,S/AS01. We searched PubMed, without language restrictions, from inception until Nov 23, 2018, using the Medical Subject Headings (MeSH) terms: ("malaria vaccines"[MeSH Terms] AND "follow-up studies"[MeSH Terms]) or the MeSH terms ("malaria"[MeSH Terms] OR "malaria vaccines"[MeSH Terms] OR "malaria, cerebral"[MeSH Terms] OR "acute malaria"[Supplementary Concept]) AND ("RTS,S-AS01E vaccine"[Supplementary Concept] OR "RTS,S-AS01B

vaccine"[Supplementary Concept] OR "RTS,S-AS01 vaccine"[Supplementary Concept]). Among a total of 95 articles retrieved and screened, no additional study with relevant data on 7 years of follow-up after RTS,S/AS01 vaccination was identified.

Added value of this study

This extension of the initial phase 3 RTS,S/AS01 efficacy study in three trial sites with different malaria transmission settings provided additional follow-up for monitoring long-term malaria incidence over a total of 7–8 years after receiving three or four doses of RTS,S/AS01.

Implications of all the available evidence

In this extension study, we observed a rebound effect in one of the trial sites during the additional 3 years of follow-up, confirming that vaccination with RTS,S/AS01 might lead to periods of increased risk to uncomplicated malaria when the protection provided by vaccination has waned. In line with epidemiological data, the incidence of severe malaria was low and declined over time as children grew older. No evidence of a rebound effect of severe malaria was documented. Careful monitoring of routine use during ongoing pilot implementations should provide further information on the overall vaccine effect.

Current interventions recommended by WHO to prevent malaria include sleeping under insecticide-treated mosquito nets, indoor residual spraying, larviciding, intermittent preventive treatment of malaria in pregnancy and infancy, and seasonal malaria chemoprevention.^{3,4} Although these methods have contributed to the marked decline in number of malaria cases, complementary tools are needed.³ In this context, the RTS,S/AS01 vaccine was developed to further control and reduce malaria incidence in children. It consists of part of the circumsporozoite protein of *Plasmodium falciparum* fused to hepatitis B surface antigen (HBsAg), co-expressed with free HBsAg in yeast, and formulated with the AS01 adjuvant.⁵ In a phase 3 study^{6–9} done in seven sub-Saharan countries, vaccine efficacy of three and four doses of RTS,S/AS01 in children who started vaccination at 5–17 months of age (older age category) were 28·3% (95% CI 23·3–32·9) and 36·3% (31·8–40·5), respectively, against clinical malaria, and 1·1% (–23·0 to 20·5) and 32·2% (13·7–46·9), respectively, against severe malaria, during an average 48-month follow-up period.^{6–9} In children who started vaccination at 6–12 weeks of age (younger age category), vaccine efficacy of three and four doses of RTS,S/AS01 were 18·3% (11·7–24·4) and 25·9% (19·9–31·5), respectively, against clinical malaria, and 10·3% (–17·9 to 31·8) and 17·3% (–9·4 to 37·5), respectively, against severe malaria, during an average 38-month follow-up period.^{6–9} Vaccine efficacy of three or four RTS,S/AS01 doses against severe and clinical malaria decreased over time in both age categories.

The results of this study have led to a positive regulatory assessment from the European Medicines Agency under the article 58 procedure in July, 2015.¹⁰ Since then, WHO has recommended pilot implementation of RTS,S/AS01 in regions with moderate-to-high malaria transmission according to an initial three-dose series, with the first dose administered as soon as possible after 5 months of age, at least 4 weeks between doses, and the third dose completed by 9 months of age, followed by a fourth dose given 15–18 months after the third dose.³

In a previous phase 2, single-centre study,¹¹ vaccine efficacy of three RTS,S/AS01 doses against clinical malaria was 35·9% (95% CI 8·1–55·3) in the first year in children aged 5–17 months, but it decreased to 4·4% (–17·0 to 21·9) over a 7-year follow-up period. A negative vaccine efficacy was measured in the fifth year in children with an increased malaria-exposure index, based on the prevalence of malaria among residents within a 1 km radius of the child's home. These results suggest that initial protection provided by three RTS,S/AS01 doses might be offset by rebound in later years in areas with high exposure to malaria.

In this extension study of the phase 3 efficacy trial, we investigated the incidence of severe and clinical malaria up to 6 or 7 years postvaccination in children from three study sites in Tanzania, Kenya, and Burkina Faso. We also collected additional immunogenicity and safety data, and recorded meningitis and cerebral malaria cases, which had emerged as safety signals in the initial study.³

Methods

Study design and participants

This open extension study to the phase 3, randomised, controlled efficacy study (NCT00866619) was done in three of the 11 sites included in the initial study, in Tanzania, Kenya, and Burkina Faso. According to the results of a previous epidemiological study based on cross-sectional surveys done in 2011–13, the three study sites had different malaria transmission intensities: *P falciparum* prevalence among children aged 6 months to 4 years ranged from 1·0% to 4·6% in Korogwe (Tanzania), 22·8% to 43·8% in Kombewa (Kenya), and 52·5% to 67·7% in Nanoro (Burkina Faso).¹² The extension study should have started at the end of the initial study (December, 2013), but study initiation was delayed by 21 months in Korogwe and 24 months in Kombewa because of delays in securing study approval, and 11 months in Nanoro for administrative reasons.

Study participants were eligible if they were enrolled in the initial study at one of the three participating sites, received at least one vaccine dose, did not withdraw, and parents provided written informed consent and, in the opinion of the investigator, would comply with the protocol requirements. Exclusion criteria were children in care, or use (or planned use) of an investigational or non-registered product during the study period. Study protocols were approved by national ethics committees and regulatory authorities of Tanzania, Kenya, and Burkina Faso. This trial was done in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. Informed consent was obtained from parents or legally authorised representatives of all children in the primary study and before any study-specific procedure in this extension study. A protocol summary is available online.

Randomisation and masking

In the initial study, children from both age categories were randomly assigned (1:1:1) by computer-generated block randomisation with minimisation by centre to receive four RTS,S/AS01 doses (four-dose group); three RTS,S/AS01 doses followed by a single dose of meningococcal serogroup C conjugate vaccine (Menjugate, Novartis, Basel, Switzerland; three-dose group); or four doses of comparator vaccines at study months 0, 1, 2, and 20 (control group).¹³ This randomisation was kept for the extension study. In the older age category (5–17 months), children in the control group received three doses of rabies vaccine (Verorab, Sanofi Pasteur, Paris, France) followed by a single dose of meningococcal serogroup C conjugate vaccine. In the younger age category (6–12 weeks), infants in the control group received four doses of meningococcal serogroup C conjugate vaccine. No additional randomisation was done and no study vaccines were administered in this extension study, which collected data since December, 2013, on average 4 years postprimary vaccination in the older children group and 3·5 years postprimary vaccination in the younger children group.

Procedures

Data collection was foreseen to continue in the same manner as in the initial trial.⁹ However, as study initiation was delayed, the extension study was set up to allow for retrospective data collection from medical charts in the routine health services to account for the period between the end of the initial study and the start of the extension study. Also, primary case definitions of clinical and severe malaria, which relied on protocol-specific procedures and were not part of the standard clinical practice,¹³ were adapted to allow retrospective events to contribute to the endpoints.

At least 0·5 mL of whole blood was drawn from all children at annual follow-up visits (2014–16) for parasite density, haemoglobin concentration, and anti-circumsporozoite antibody concentration measurement. Antibody concentrations against *P falciparum* circumsporozoite-repeat region (antigen R32LR) were measured by a standard ELISA.¹⁴ The assay cutoff was changed from 0·5 ELISA unit (EU) per mL in the initial study to 1·9 EU/mL in this extension study because of revalidation with new assay reagents. Serious adverse events of interest were captured at the health-care facilities or by direct questioning at the annual visits.

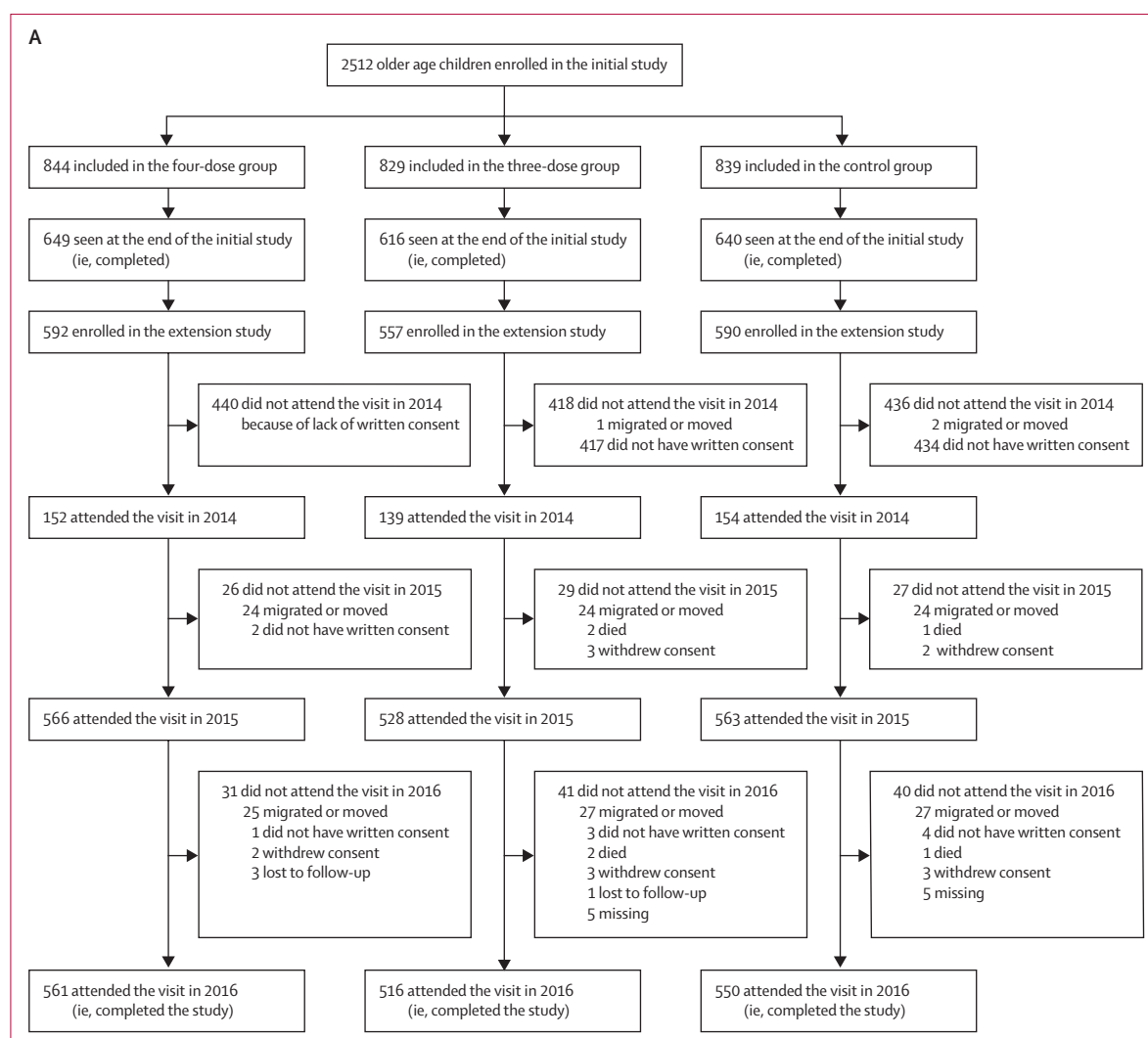
Outcomes

The primary objective of this extension study was long-term severe malaria incidence over the additional 3-year follow-up period (January, 2014, to December, 2016) across transmission settings in both age categories. The case definition for severe malaria (appendix p 2) was evidence of *P falciparum* asexual parasitaemia (>5000 parasites per μ L) with at least one of the previously described marker of disease severity⁹ or a parasitologically confirmed malaria serious adverse event report including any malaria-related preferred terms (malaria, *P falciparum* infection, cerebral malaria). The case definition for cerebral malaria was evidence of *P falciparum* asexual parasitaemia with either a Blantyre score of 2 or less or a malaria serious adverse event report including cerebral malaria as preferred term. Vaccine efficacy was calculated to explore the resultant differences in severe malaria incidence between groups.

Secondary objectives were the description of severe malaria incidence over 6–7 years since the start of the initial study in 2009. We also evaluated incidences of clinical malaria and malaria hospitalisation (either admission to hospital or treatment in hospital) over the 3 additional follow-up years and during the entire follow-up. Clinical malaria cases were defined as cases with evidence of *P falciparum* asexual parasitaemia with fever at the time of presentation, or history of fever within 24 h of presentation in a child who was unwell and brought to a health-care facility for treatment. The malaria hospitalisation case definition was a hospitalisation for which, in the judgment of the clinician,

See Online for appendix

For the study (number 200599) protocol see <http://www.gsk-clinicalstudyregister.com>



(Figure 1 continues on next page)

P. falciparum infection was the sole or a major contributing factor to the presentation. *P. falciparum* asexual parasitaemia was defined as either a positive blood film (double or single slide reading) or a positive rapid diagnostic test. Secondary objectives also included the evaluation of prevalent parasitemia (presence of *P. falciparum* asexual parasites in a blood film), and moderate (haemoglobin concentration <0.8 g/L) and severe (haemoglobin concentration <0.5 g/L) anaemia at annual cross-sectional timepoints. Serious adverse events of interest (fatalities, serious adverse events related to vaccination or to study participation, malaria hospitalisation, potential immune-mediated diseases [pIMDs, defined in appendix pp 3, 4], and meningitis) were collected and analysed according to MedDRA Preferred Term level. Anti-circumsporozoite antibody responses were evaluated at annual cross-sectional timepoints.

Statistical analysis

The primary objectives of this extension study are descriptive; therefore, no formal sample size calculation was done for these endpoints. All results were presented for the modified intention-to-treat population, including all participants from the three sites who received at least one dose of study vaccine.

Baseline demographic characteristics (age at enrolment in the initial and extension studies and sex) were evaluated by group and age category. Categorical variables were presented as percentages and numerical variables as mean values.

In this study, malaria incidence is defined as the number of episodes divided by the follow-up time, and was calculated for each age category over defined risk periods (appendix p 5). Vaccine efficacy is defined as 1-incidence ratios (incidence in the four-dose or three-dose group over incidence in the control group). Vaccine efficacy was

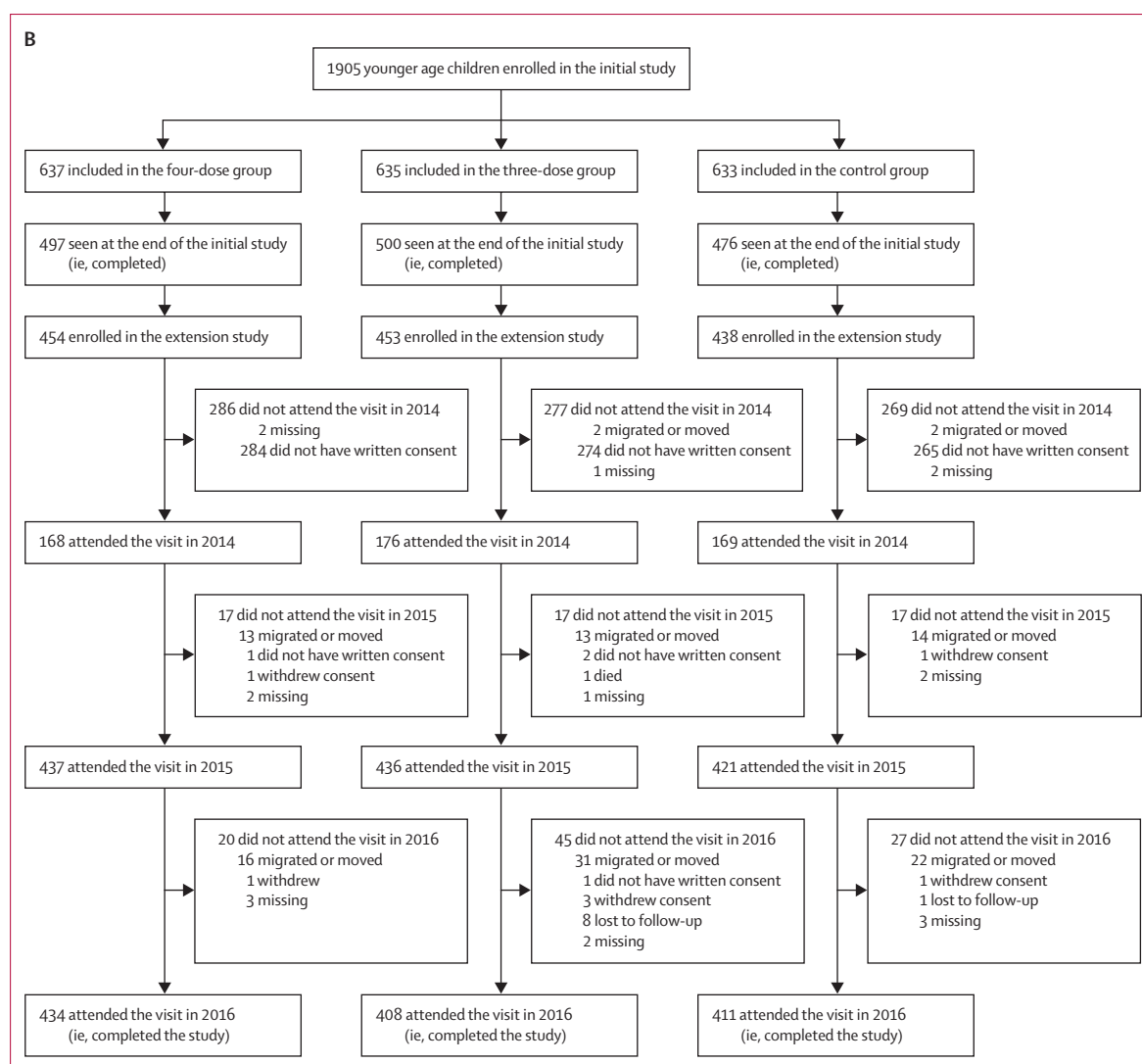


Figure 1: Trial profile for children in the older age category (A) and the younger age category (B)

Older age category included children aged 5–17 months; younger age category included infants aged 6–12 weeks. Some participants who received at least one dose of vaccine in the initial study and had completed it were not enrolled in the extension study because of loss to follow-up, were not willing to participate in the extension study, withdrew consent during the initial study, or died.

calculated by a negative binomial regression with time at risk as offset variable, allowing for interdependence between episodes within the same participant. Vaccine efficacy estimates were presented with 95% CIs and p values. For prevalent endpoints (parasitaemia and anaemia), overall reductions were estimated as 1-risk ratios (proportion of children reporting events in the four-dose or three-dose group over proportion of children reporting events in the control group) and were presented with 95% CIs and p values. Sensitivity analyses excluding the endpoints and follow-up time collected retrospectively were done for severe and clinical malaria over the 3-year follow-up period and the entire 6–7-year study period.

Proportions of children with fatal serious adverse events, vaccine-related serious adverse events, malaria

hospitalisations, pIMDs, and meningitis were collected by study group. Anti-circumsporozoite antibody geo-metric mean concentrations were calculated with exact 95% CIs.

Statistical analyses were done with SAS (version 9.2). The trial was overseen by a data monitoring committee, reviewing the safety data of the trial independently from the sponsor or the investigators, and making recommendations on the continuation of the trial. The study is registered with ClinicalTrials.gov, number NCT02207816.

Role of the funding source

GlaxoSmithKline Biologicals SA was involved in study design, and coordinated data collection, data analysis, data interpretation, and writing of the report. The PATH

	Four-dose group	Three-dose group	Control group
Older age category			
11 sites at the start of the initial study			
Age, months	10·7 (3·8)	10·6 (3·8)	10·6 (3·7)
Sex			
Male	1509/2976 (51%)	1472/2972 (50%)	1471/2974 (49%)
Female	1467/2976 (49%)	1500/2972 (50%)	1503/2974 (51%)
Three sites at the start of the initial study			
Age, months	10·4 (3·9)	10·4 (3·8)	10·4 (3·8)
Sex			
Male	415/844 (49%)	407/829 (49%)	415/839 (49%)
Female	429/844 (51%)	422/829 (51%)	424/839 (51%)
Three sites at the start of the extension study			
Age, years	5·9 (0·7)	5·9 (0·7)	5·9 (0·7)
Sex			
Male	289/592 (49%)	288/557 (52%)	295/590 (50%)
Female	303/592 (51%)	269/557 (48%)	295/590 (50%)
Younger age category			
11 sites at the start of the initial study			
Age, weeks	7·2 (1·4)	7·1 (1·4)	7·1 (1·4)
Sex			
Male	1116/2180 (51%)	1118/2178 (51%)	1079/2179 (50%)
Female	1064/2180 (49%)	1060/2178 (49%)	1100/2179 (50%)
Three sites at the start of the initial study			
Age, weeks	6·9 (1·5)	6·7 (1·4)	6·8 (1·4)
Sex			
Male	323/637 (51%)	336/635 (53%)	309/633 (49%)
Female	314/637 (49%)	299/635 (47%)	324/633 (51%)
Three sites at the start of the extension study			
Age, years	4·4 (0·6)	4·4 (0·6)	4·4 (0·5)
Sex			
Male	240/454 (58%)	242/453 (53%)	218/438 (50%)
Female	214/454 (42%)	211/453 (47%)	220/438 (50%)

Data are mean (SD) or n/N (%). Four-dose group: children who received four doses of RTS,S/AS01 on months 0, 1, 2, and 20 of the initial study. Three-dose group: children who received three doses of RTS,S/AS01 on months 0, 1, 2 and one dose of comparator vaccine at month 20 on the initial study. Control group: children who received four doses of a comparator vaccine on months 0, 1, 2, and 20 of the initial study.

Table: Demographic characteristics of participants from the 11 sites included at the start of the initial study and from the three sites included in the extension study (at the start of the initial and extension studies; enrolled population)

Malaria Vaccine Initiative contributed to the initial study design and data interpretation, but was not involved in data collection, data analysis, or writing of the report for this extension follow-up. The authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

At the three participating sites, the initial study enrolled 2512 children in the older age category and 1905 children

in the younger age category (figure 1). Of these, 1739 older and 1345 younger children were enrolled in the extension study, and 1627 older children and 1253 younger children attended the study visit in 2016 (ie, completed the trial). The main reason for not attending the last visit was migration.

Demographic characteristics at the start of the extension study and the initial trial were well matched between groups in both age categories (table). At the start of the extension study, the mean age was 5·9 years (SD 0·7) in the older age category and 4·4 years (0·6) in the younger age category (table).

In the extension study, 66 severe malaria cases were reported during the 3-year follow-up, resulting in severe malaria incidences in the four-dose (older age category 0·0040 cases per person year at risk [PPY; 95% CI 0–0·033]; younger age category 0·0070 PPY [0·0010–0·058]), three-dose (0·0070 PPY [0·0010–0·052]; 0·0070 PPY [0·0010–0·054]), and control groups (0·0090 PPY [0·0010–0·066]; 0·011 PPY [0·0010–0·083]; figure 2, appendix p 6). The overall decrease in severe malaria incidence over time observed in all groups in both age categories was driven by the results obtained in Kombewa and Nanoro, because of the high severe malaria incidence in the initial study in these sites (figure 2). Vaccine efficacy against severe malaria during the 3-year follow-up did not contribute significantly to the overall efficacy (older children: 53·7% [–13·7 to 81·1], $p=0·093$ for the four-dose group and 23·3% [–67·1 to 64·8], $p=0·50$ for the three-dose group; younger children 32·1% [–53·1 to 69·9], $p=0·35$ for the four-dose group and 37·6% [–44·4 to 73·0], $p=0·27$ for the three-dose group). Vaccine efficacy against severe malaria over the entire follow-up was 36·7% (14·6 to 53·1; $p=0·0028$) for the four-dose group and 10·1% (–18·1 to 31·6; $p=0·44$) for the three-dose group for older children, and 31·0% (4·7–50·0; $p=0·025$) for the four-dose and 34·2% (8·7–52·6; $p=0·012$) for the three-dose schedules for younger children (appendix pp 7–9). Two cases of cerebral malaria were reported in the older children, both in the three-dose group, and one case in the younger children, in the four-dose group.

During the 3-year follow-up, clinical malaria incidences in the four-dose, three-dose, and control groups were 1·079 PPY (95% CI 0·152–7·662), 1·108 PPY (0·156–7·868), and 1·016 PPY (0·143–7·213), respectively, in the older children group, and 1·632 PPY (0·23–11·59), 1·563 (0·22–11·104), and 1·686 (0·237–11·974) PPY, respectively, in the younger children group (figure 3, appendix p 10). In both age categories, clinical malaria incidence was lower in Korogwe (range 0·165–0·368 PPY) than in Kombewa (1·160–1·418 PPY) and Nanoro (1·998–3·124 PPY). Clinical malaria incidence tended to be lower during the 3-year extension study than during the initial study in Kombewa and Nanoro. Comparing the incidences between treatment groups, no additional benefit of vaccination was observed during the 3-year follow-up. In the older children category, vaccine efficacy

against clinical malaria was -5.3% (95% CI -20.0 to 7.7 , $p=0.44$) for the four-dose group and -8.1% (-23.9 to 5.7 , $p=0.26$) for the three-dose group during the 3-year follow-up, and 23.7% (15.9 – 30.7 , $p<0.0001$) for the four-dose group and 19.1% (10.8 – 26.7 ; $p<0.0001$) for the three-dose group during the entire follow-up (figure 4, appendix pp 11, 12). In Nanoro, there were more cases of clinical malaria in RTS,S/AS01 recipients than there were in the control group (four-dose vaccine efficacy -30.3% [95% CI -59.5 to -6.4], $p=0.011$; three-dose vaccine efficacy -26.0% [-56.0 to -1.8], $p=0.034$; appendix p 11, 12). However, there was still a benefit of vaccination during the entire 7-year follow-up in Nanoro, with a vaccine efficacy of 13.8% (3.3 – 23.1 ; $p=0.012$) for the four-dose schedule and 7.2% (-4.2 to 17.5 ; $p=0.21$) for the three-dose schedule. In the younger children, there were no significant differences between groups in terms of clinical malaria incidence during the three-year follow-up (figure 4, appendix pp 11, 12).

Sensitivity analyses, excluding follow-up time and episodes occurring within the retrospective data collection period, showed similar results in terms of severe and clinical malaria incidences to analyses including both retrospective and prospective data (appendix pp 13, 14).

Because most severe malaria cases reported in this extension study also met the case definition for malaria hospitalisation, results were similar (data not shown).

Prevalent parasitaemia was measured at annual cross-sectional assessments in Nanoro in 2014, and in the three study sites in 2015 and 2016 (figure 5, appendix pp 15–17). Across both age categories and all groups, a higher proportion of children with prevalent parasitaemia was observed in Nanoro compared with Kombewa and Korogwe, although this difference was not statistically significant. In Nanoro, older children who received three or four RTS,S/AS01 doses showed lower prevalent parasitaemia than did those in the control group (appendix p 15). In 2014, reductions in prevalent parasitaemia were 40.8% (95% CI 15.7 – 58.8 ; $p<0.0001$) for the four-dose schedule and 42.7% (17.4 – 60.8 ; $p<0.0001$; appendix pp 16, 17) for the three-dose schedule in this site. In 2015 and 2016, reductions in prevalent parasitaemia were non-significant. No significant reduction in prevalent parasitaemia was observed in the two other study sites, nor in the younger children in Nanoro.

In both age categories, prevalence of moderate anaemia decreased with time and was less than 10% at any site and timepoint. In older children, 61 cases of anaemia (60 moderate and one severe [in 2015]) were reported during the three cross-sectional assessments. In younger children, 98 cases of anaemia were reported (96 moderate and two, in the three-dose group, severe [one in 2015 and one in 2016]). No differences between study groups were observed.

In both age categories, no vaccine-related severe adverse events and no pIMDs were reported during the additional 3 years of follow-up. In the older children

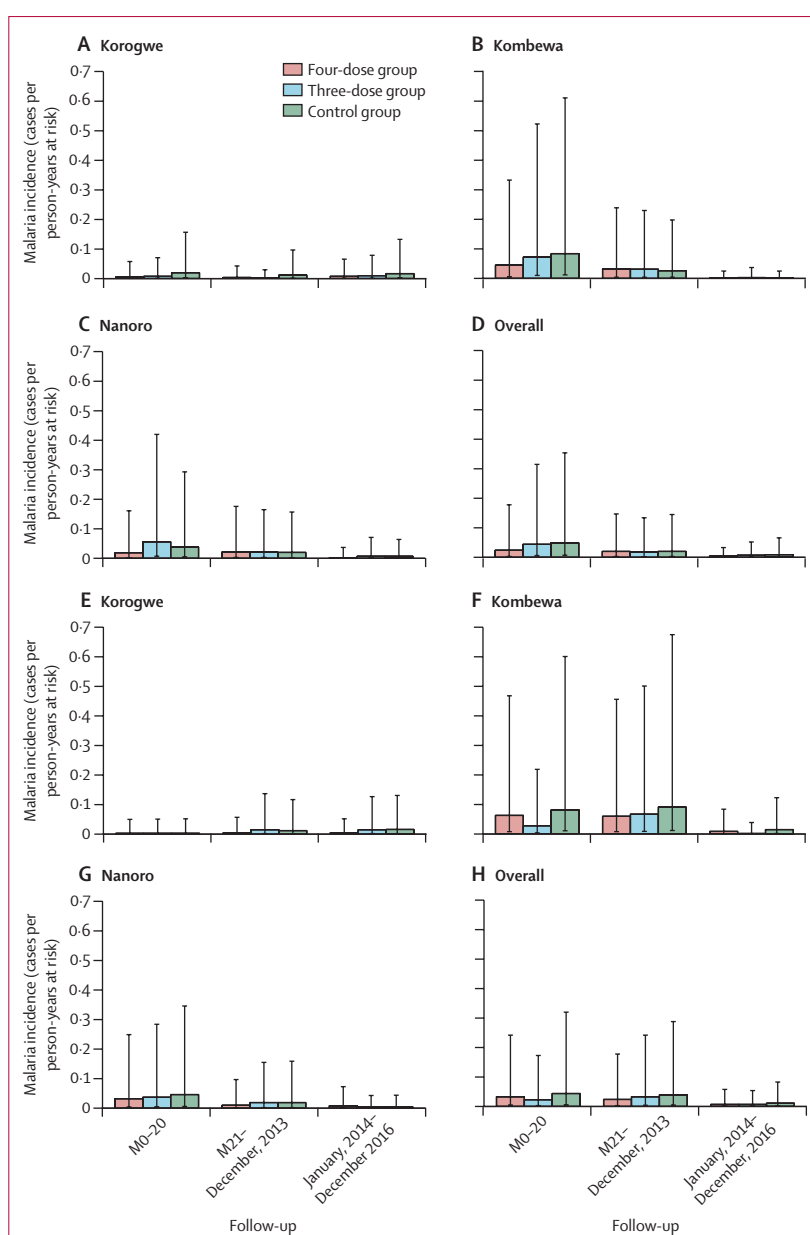


Figure 2: Incidence of severe malaria in children from the older age category (A–D) and the younger age category (E–H) in the intention-to-treat population (A, E) Korogwe. (B, F) Kombewa. (C, G) Nanoro. (D, H) Overall. Older age category included children aged 5–17 months; younger age category included infants aged 6–12 weeks. M0=time of the first dose administration in the initial study. M20=20 months after the first dose in the initial study. M21=21 months after the first dose in the initial study. Error bars represent 95% CIs.

group, serious adverse events associated with malaria were reported for 19 children in the four-dose group, 20 children in the three-dose group, and 24 children in the control group. Fatal severe adverse events were reported in two children (one boy and one girl) in the four-dose group, seven children (three boys and four girls) in the three-dose group, and five children (two boys and three girls) in the control group. One child from the four-dose group, two children from the three-dose

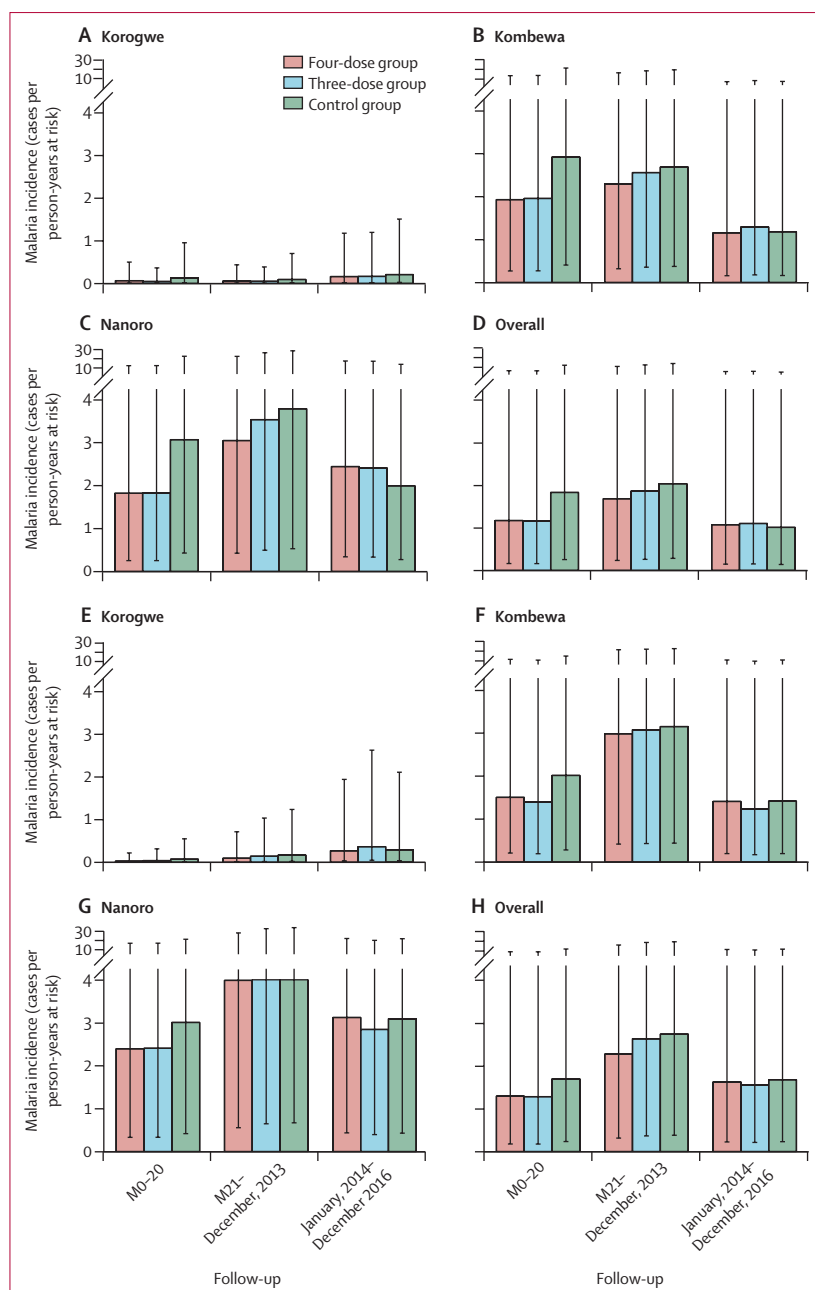


Figure 3: Incidence of clinical malaria in children from the older age category (A–D) and the younger age category (E–H) in intention-to-treat population

(A, E) Korogwe. (B, F) Kombewa. (C, G) Nanoro. (D, H) Overall. Older age category included children aged 5–17 months; younger age category included infants aged 6–12 weeks. M0=time of the first dose administration in the initial study. M20=20 months after the first dose in the initial study. M21=21 months after the first dose in the initial study. Error bars represent 95% CIs.

group, and two children from the control groups died with a malaria diagnosis. In younger children, severe adverse events associated with malaria were reported for 17 children in the four-dose group, 16 children in the three-dose group, and 24 children in the control group. Fatal severe adverse events were reported in three children (three girls) in the four-dose group, three children

(one boy and two girls) in the three-dose group, and two children (one boy and one girl) in the control group. Of these, two children from the four-dose group, one child from the three-dose group, and one child from the control group died with a malaria diagnosis. In the older children group, one meningococcal meningitis case was reported in a child from the control group, in Nanoro. In the younger children, four meningitis cases were reported: one suspected meningitis in a child from the four-dose group in Kombewa, one meningococcal meningitis in a child from the four-dose group in Nanoro, and one acute bacterial meningitis and one possible meningitis in children from the control group in Kombewa.

In the older age category, 95.5% of children in the four-dose group and 82.8% of children in the three-dose groups had anti-circumsporozoite antibody concentrations of 1.9 EU/mL or more at the cross-sectional assessment in 2016 (figure 6A). In the younger age category, 67.2% of children in the four-dose group and 52.8% of children in the three-dose group had anti-circumsporozoite antibody concentrations of 1.9 EU/mL or more in 2016 (figure 6B). In both age categories, anti-circumsporozoite antibody geometric mean concentrations remained low during the entire study period in the control group (figure 6).

Discussion

Overall, this extension study showed that severe malaria incidence declined in children up to 6 or 7 years after RTS,S/AS01 vaccination, regardless of the treatment assignment. This finding was expected for children aged 5–8 years since severe malaria incidence declines over time because children are exposed to the malaria parasite when they grow older.¹⁵ Also, during the 3-year follow-up, we observed a slightly higher severe malaria incidence in the lower transmission setting (Korogwe) than in the higher transmission settings (Nanoro and Kombewa). This finding might be explained by the earlier natural acquisition of immunity in areas with higher malaria transmission intensities, leading to peaks in severe malaria incidence at older ages in lower transmission settings. Comparing the study groups, for both age categories and both vaccination schedules, there was no significant vaccine efficacy against severe malaria over the additional 3 years of follow-up, nor was there any evidence of increased susceptibility in RTS,S/AS01 recipients compared with the control group (rebound). Nevertheless, positive, although not always significant, vaccine efficacy against severe malaria was noted over the entire 6 years or 7 years of follow-up. These findings are mostly explained by the initial efficacy offered by the vaccine at earlier timepoints in the initial study,⁹ since both vaccine efficacy and incidence of severe malaria subsequently declined. Another interesting observation was that over the entire 6-year follow-up, we noted a significant vaccine efficacy against severe malaria for

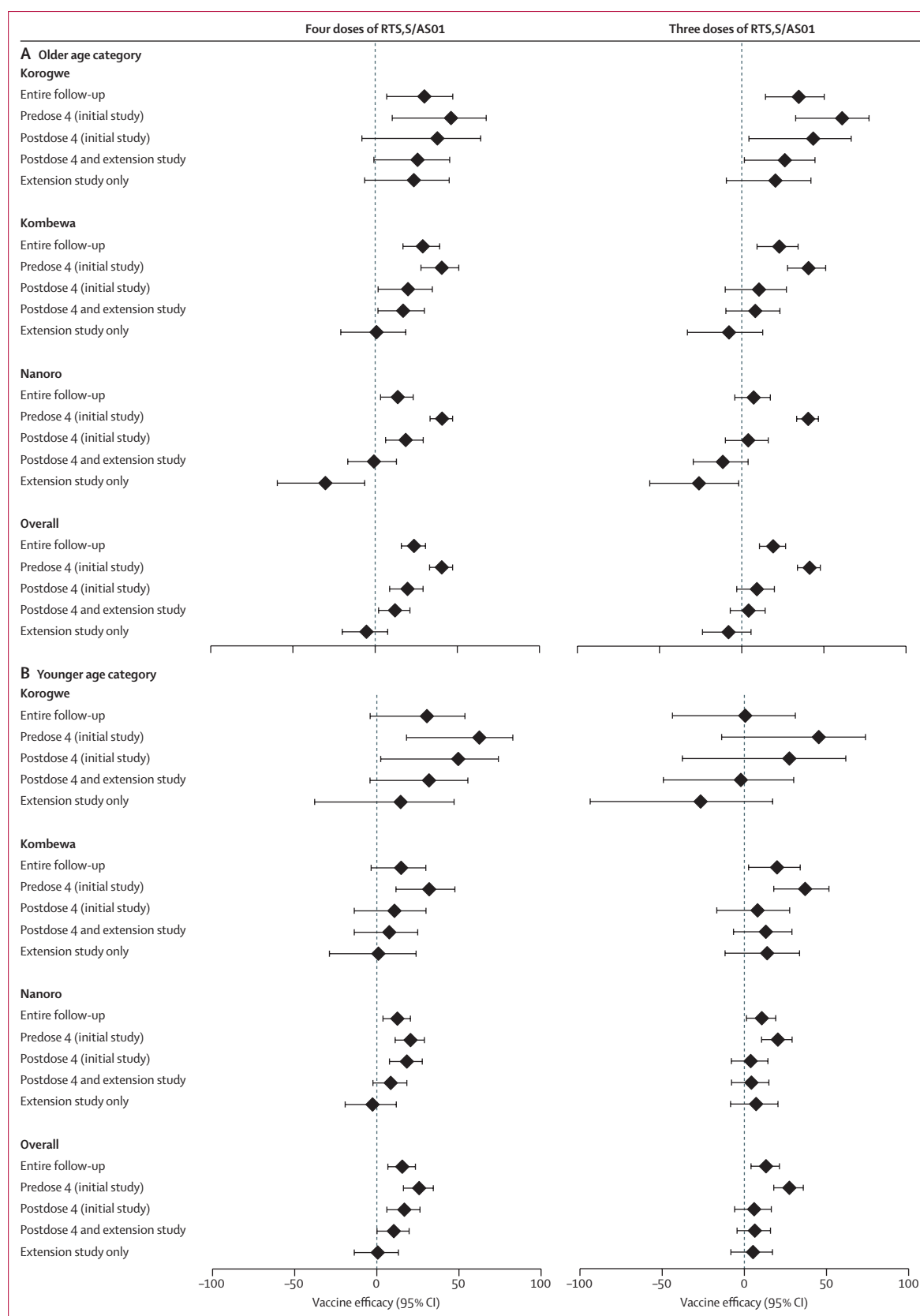


Figure 4: Vaccine efficacy against clinical malaria (overall and per study site) in children from both age categories who received three or four doses of RTS,S/AS01 (intention-to-treat population)
Older age category included children aged 5–17 months; younger age category included infants aged 6–12 weeks.

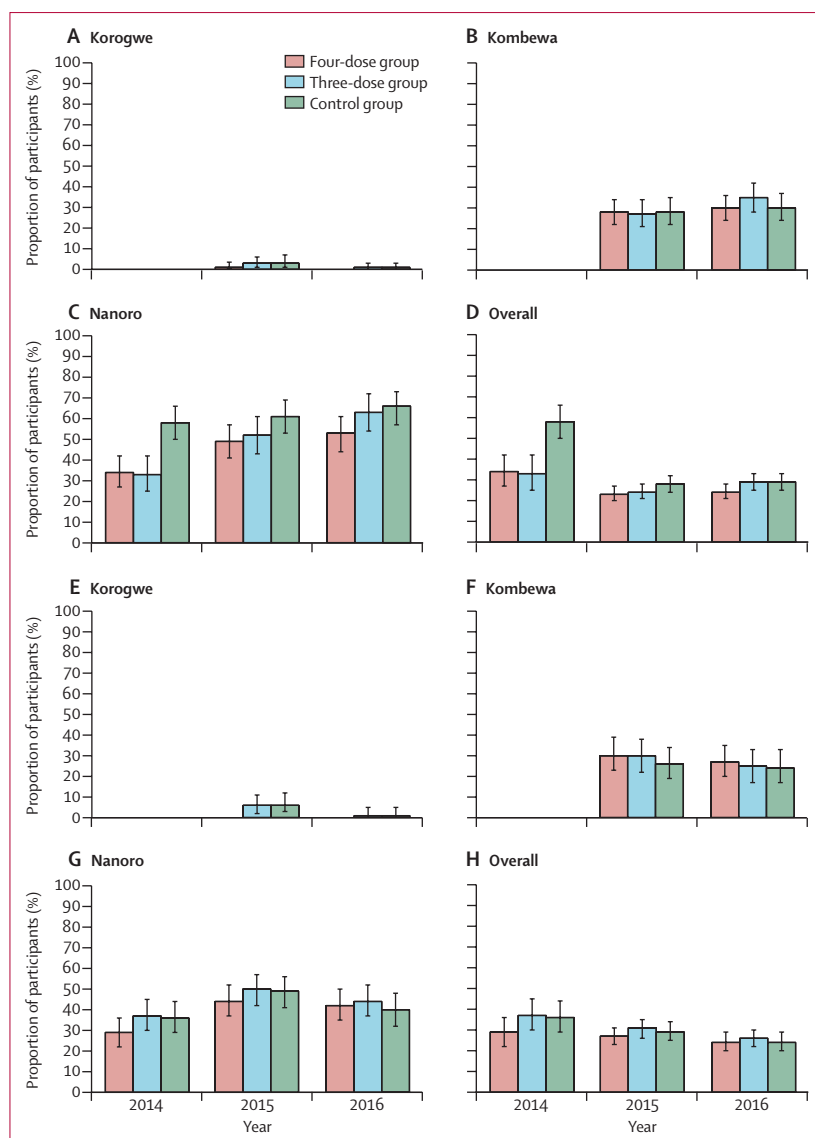


Figure 5: Proportions of children with prevalent parasitemia at the cross-sectional assessment in 2014, 2015, and 2016 in the older age category (A–D) and the younger age category (E–H)

(A, E) Korogwe. (B, F) Kombewa. (C, G) Nanoro. (D, H) Overall. Older age category included children aged 5–17 months; younger age category included infants aged 6–12 weeks. Error bars represent 95% CIs.

both the three-dose and the four-dose vaccination schedules in the younger age category, which was not documented after three doses in the initial trial.⁹ Although an explanation could be that we did not include all the 11 sites from the initial trial in this extension study, it might also suggest that in the longer term, vaccine efficacy against severe malaria could be achieved when vaccinating 6–12-month-old infants.

During the additional follow-up, clinical malaria incidence remained high in the two sites with a high malaria transmission intensity. Comparing the treatment groups, overall vaccine efficacy against clinical malaria decreased over time, with no evidence of significant vaccine efficacy for either schedules during the additional

3 years of follow-up. In the older children group, vaccine efficacy against clinical malaria was even significantly negative in this extension study for both schedules in Nanoro, Burkina-Faso. This observation is in line with the previous phase 2 study¹¹ done in Kenya, suggesting that initial protection provided by three RTS,S/AS01 doses was offset by rebound after 5 years in areas with higher than average exposure to malaria parasites. The hypothesis of a potential rebound effect in Nanoro was supported by the lower incidence of asymptomatic prevalent parasitaemia in RTS,S/AS01 recipients than in the control group in the absence of vaccine efficacy against malaria. This effect suggests that due to delayed natural acquisition of immunity, vaccine recipients could be less likely to be asymptomatically infected compared with controls and, therefore, more likely to be diagnosed and treated when the vaccine induced immunity has waned. Nevertheless, the increase in clinical malaria incidence in the RTS,S/AS01 groups at older age in Nanoro did not outweigh the initial benefit, and significant vaccine efficacy against clinical malaria was documented over the whole trial duration. Moreover, this period of increased risk for clinical malaria was not accompanied by, nor resulted in, an increased risk for severe malaria or a shift towards cerebral malaria.

No imbalance in the number of meningitis cases with any cause was observed in this extension study.^{3,8,9} Only three cerebral malaria cases, which are known to be more common in older children than during the first 2 years of life,¹⁶ were reported in this extension study. The low number of meningitis and cerebral malaria cases reported during the additional 3-year follow-up is reassuring in light of the safety signals observed in the initial study. No new safety signal was identified, nor any other compelling evidence that would require changing the phase 4 studies and pilot implementation of RTS,S/AS01.

During the 3-year follow-up, anti-circumsporozoite antibody concentrations declined in vaccine recipients, but remained above baseline concentrations. In the absence of significant vaccine efficacy, these results suggest that the protection provided by the low concentration of anti-circumsporozoite antibodies, in addition to the natural immunity already acquired in the RTS,S/AS01 recipients, did not show additional benefit compared with the natural immunity acquired by the children in the control group.

Extending the initial phase 3 study was the fastest and most robust way to detect changes in malaria susceptibility, to assess the possibility of a rebound following administration of three or four doses of RTS,S/AS01, and to evaluate whether the administration of a fourth dose of RTS,S/AS01 changed the long-term outcomes. However, this study has several limitations, including its delayed initiation and the retrospective data collection between the end of the initial trial and the start of the extension study, which could have affected the study power and accuracy. To minimise under-reporting, the study monitoring plan was modified to

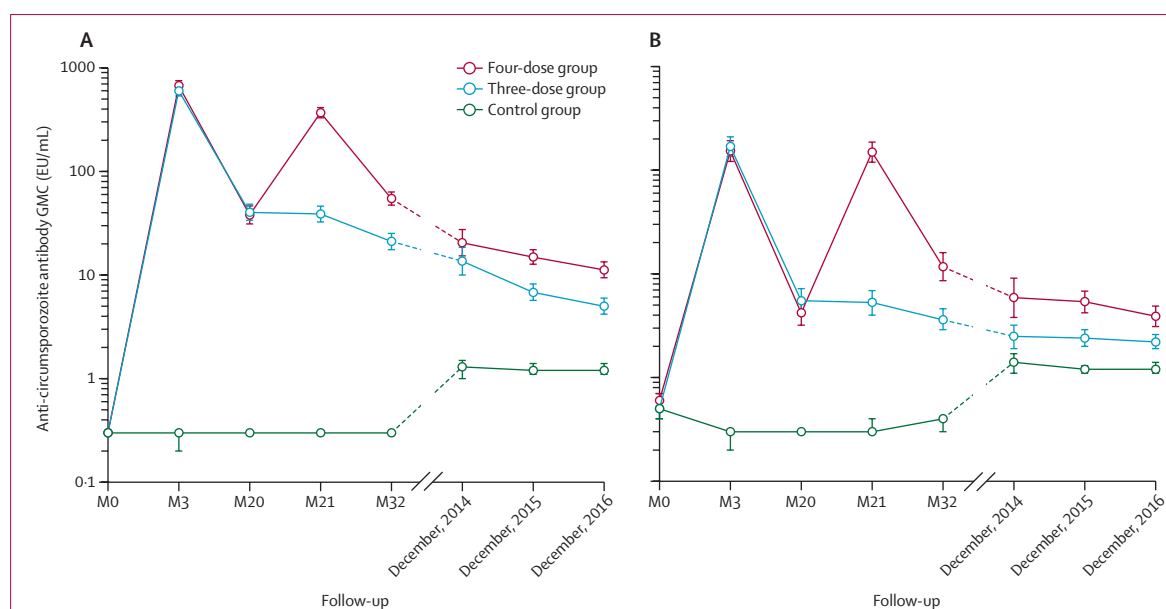


Figure 6: Anti-circumsporozoite-repeat region antibody kinetics (evolution of antibody geometric mean concentrations over time) in the older age category (A) and the younger age category (B) in the intention-to-treat population

Data shown in a logarithmic scale. Error bars represent 95% CIs. Older age category included children aged 5–17 months; younger age category included infants aged 6–12 weeks. The anti-circumsporozoite ELISA assay used in the initial study (up to month 32; cutoff=0.5 ELISA units [EU]/mL) was different from the newly validated anti-circumsporozoite ELISA used in this extension study (years 5, 6, and 7; cutoff=1.9 EU/mL). The apparent increase of anti-circumsporozoite antibody geometric mean concentrations (GMCs) in the control groups before the extension study is likely to be an artefact of the higher cutoff of the new anti-circumsporozoite ELISA assay used, although some natural acquisition of anti-circumsporozoite antibodies might also have occurred. GMC=geometric mean concentration. EU=ELISA unit. M=month.

account for all possible missed cases. Malaria case definitions were modified to allow any evidence of recent or ongoing *P. falciparum* infection (any positive microscopic slide reading or positive rapid diagnostic test), leading to less specific case definitions but allowing maintenance of sensitivity and maximising the probability to include all potential malaria events. Sensitivity analyses excluding the retrospective data collection period were done to explore the effect of the delayed study initiation. Although these sensitivity analyses resulted in the same conclusions as the main analyses, these results should be interpreted cautiously for severe malaria because of the limited number of cases. However, the probability of under-reporting was lower for severe malaria cases than for clinical malaria cases, since severe malaria cases are based on inpatient hospital records. Other drawbacks were the loss of participants from the initial trial who were not included in this extension study, and the low number of participants who attended the annual visit in 2014, leading to a paucity of immunogenicity and parasite prevalence data for this timepoint. Also, the fact that the trial was done in an open way could potentially have affected the physicians' judgement.

In conclusion, we showed that the incidence of severe malaria substantially declined over time and was low in all study groups in this long-term extension study of a phase 3 trial. Although rebound against clinical malaria was observed in the older children group in one of the

study centres with high malaria prevalence, no rebound of severe disease was detected after either three or four vaccine doses in both age categories. No safety signal was identified during the additional 3-year follow-up.

Contributors

YGM, EJ, JL, ML, LO, WO, and HT designed the study. SG, JL, EL, AM, LO, WO, HS, VS, HT, DV, IV, and AW collected the data. EG contributed to the analysis. SG, YGM, EJ, JL, ML, AM, LO, WO, FR, LS, VS, and HT interpreted the data. All authors reviewed and commented on a draft version of the manuscript and gave their final approval for it to be submitted for publication.

Declaration of interests

EG, YGM, EJ, ML, FR, and LS are employees of GlaxoSmithKline (GSK) group of companies. EJ, FR, and LS hold restricted shares in the GSK group of companies. YGM and LS hold stock options in the GSK group of companies. LO declares that he received the Trust in Science grant funding from GSK group of companies. SG, EL, AM, and IV report grants from GSK group of companies during the conduct of the study. JL, WO, VS, HS, HT, DV, IV, and AW declare no competing interests.

Data sharing

Anonymised individual participant data are available and can be accessed (appendix p 1).

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