Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial

*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. Herein, we report the final results from the same trial, including the efficacy of a booster dose.

Methods From March 27, 2009, until Jan 31, 2011, children (age 5–17 months) and young infants (age 6–12 weeks) were enrolled at 11 centres in seven countries in sub-Saharan Africa. Participants were randomly assigned (1:1:1) at first vaccination by block randomisation with minimisation by centre to receive three doses of RTS,S/AS01 at months 0, 1, and 2 and a booster dose at month 20 (R3R group); three doses of RTS,S/AS01 and a dose of comparator vaccine at month 20 (R3C group); or a comparator vaccine at months 0, 1, 2, and 20 (C3C [control group]). Participants were followed up until Jan 31, 2014. Cases of clinical and severe malaria were captured through passive case detection. Serious adverse events (SAEs) were recorded. Analyses were by modified intention to treat and per protocol. The coprimary endpoints were the occurrence of malaria over 12 months after dose 3 in each age category. In this final analysis, we present data for the efficacy of the booster on the occurrence of malaria. Vaccine efficacy (VE) against clinical malaria was analysed by negative binomial regression and against severe malaria by relative risk reduction. This trial is registered with ClinicalTrials.gov, number NCT00866619.

Findings 8922 children and 6537 young infants were included in the modified intention-to-treat analyses. Children were followed up for a median of 48 months (IQR 39–50) and young infants for 38 months (34–41) after dose 1. From month 0 until study end, compared with 9585 episodes of clinical malaria that met the primary case definition in children in the C3C group, 6616 episodes occurred in the R3R group (VE 36·3%, 95% CI 31·8–40·5) and 7396 occurred in the R3C group (28·3%, 23·3–32·9); compared with 171 children who experienced at least one episode of severe malaria in the C3C group, 116 children experienced at least one episode of severe malaria in the R3R group (32·2%, 13·7 to 46·9) and 169 in the R3C group (1·1%, –23·0 to 20·5). In young infants, compared with 6170 episodes of clinical malaria that met the primary case definition in the C3C group, 4993 episodes occurred in the R3R group (VE 25·9%, 95% CI 19·9–31·5) and 5444 occurred in the R3C group (18·3%, 11·7–24·4); and compared with 116 infants who experienced at least one episode of severe malaria in the C3C group, 96 infants experienced at least one episode of severe malaria in the R3R group (17·3%, 95% CI –9·4 to 37·5) and 104 in the R3C group (10·3%, –17·9 to 31·8). In children, 1774 cases of clinical malaria were averted per 1000 children (95% CI 1387–2186) in the R3R group and 1363 per 1000 children (995–1797) in the R3C group. The numbers of cases averted per 1000 young infants were 983 (95% CI 592–1337) in the R3R group and 558 (158–926) in the R3C group. The frequency of SAEs overall was balanced between groups. However, meningitis was reported as a SAE in 22 children: 11 in the R3R group, ten in the R3C group, and one in the C3C group. The incidence of generalised convulsive seizures within 7 days of RTS,S/AS01 booster was 2·2 per 1000 doses in young infants and 2·5 per 1000 doses in children.

Interpretation RTS,S/AS01 prevented a substantial number of cases of clinical malaria over a 3–4 year period in young infants and children when administered with or without a booster dose. Efficacy was enhanced by the administration of a booster dose in both age categories. Thus, the vaccine has the potential to make a substantial contribution to malaria control when used in combination with other effective control measures, especially in areas of high transmission.

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Introduction

Substantial 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 protein of *Plasmodium falciparum*, expressed by the malaria parasite at the pre-erythrocytic stage, in which part of the circumsporozoite sequence is coexpressed with fused and free hepatitis B surface antigen[2,3] and formulated with the AS01 adjuvant. Previous studies have established the ability of RTS,S/AS01 to provide protective immunity.[4–6]

We undertook a phase 3, double-blind (observer-blind), individually randomised, controlled trial to

Research in context

Evidence before this study

We did a systematic literature search between Dec 18, 2014, and Feb 20, 2015, of randomised controlled trials of RTS,S malaria vaccine on PubMed, the Cochrane Library, and other relevant data sources for the period 1984 to Jan 31, 2015. We searched PubMed using the Medical Subject Headings (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, we used the following key search terms: "RTS,S", "malaria vaccines", AND "clinical trials". The 60 manuscripts identified included five that reported the results of randomised controlled trials with long-term safety or efficacy follow-up or booster dose, two pooled analyses, and two systematic reviews.

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. Additionally, the study showed 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 Expanded Program on Immunization vaccination. The vaccine has consistently shown a good safety profile, although a meningitis safety signal reported among older children will need further follow-up. The results of the present study show the potential public health benefit of the RTS,S vaccine as an additional means for malaria control whilst the next generation of malaria vaccines are being developed.

assess the efficacy and safety of RTS,S/AS01. Study results up to 18 months of follow-up have been reported previously.[7–9] The coprimary endpoints of efficacy to clinical malaria over the first 12 months after dose 3 were 55·8% (97·5% CI 50·6–60·4) in children aged 5–17 months and 31·3% (23·6–38·3) in infants aged 6–12 weeks.[7–9] Protection against clinical and severe malaria was noted in both children and young infants during the first 12 months after vaccination, but protection waned over time in both age categories.[9] Herein, we report the efficacy, immunogenicity, and safety of RTS,S/AS01 and the number of cases averted by the use of the vaccine in children and young infants followed up to the end of the trial, including findings in those who received a booster dose of vaccine.

Methods

Study design and participants

We undertook this phase 3, double-blind, observer-blind, individually randomised controlled trial between March 27, 2009, and Jan 31, 2014, at 11 centres in seven countries in sub-Saharan Africa that are situated in areas with different intensities of malaria transmission (appendix p 18). Trial methods have been reported previously,[7–9,10] and are described in the appendix (pp 4–13). We initially designed this trial to assess vaccine efficacy (VE), safety, and immunogenicity during 32 months of follow-up, but the protocol was amended before month 32, on Dec 1, 2010, to extend the follow-up period to Dec 31, 2013 (median follow-up 48 months for children and 38 months for young infants). Parents or legally authorised representatives of all participants provided written or thumb printed and witnessed informed consent at enrolment to the primary study and to the extension. Access to an insecticide-treated bednet was optimised for all screened children. Net use and condition were assessed during protocol-specified home visits done at month 13, month 31, and 1 month before study end.

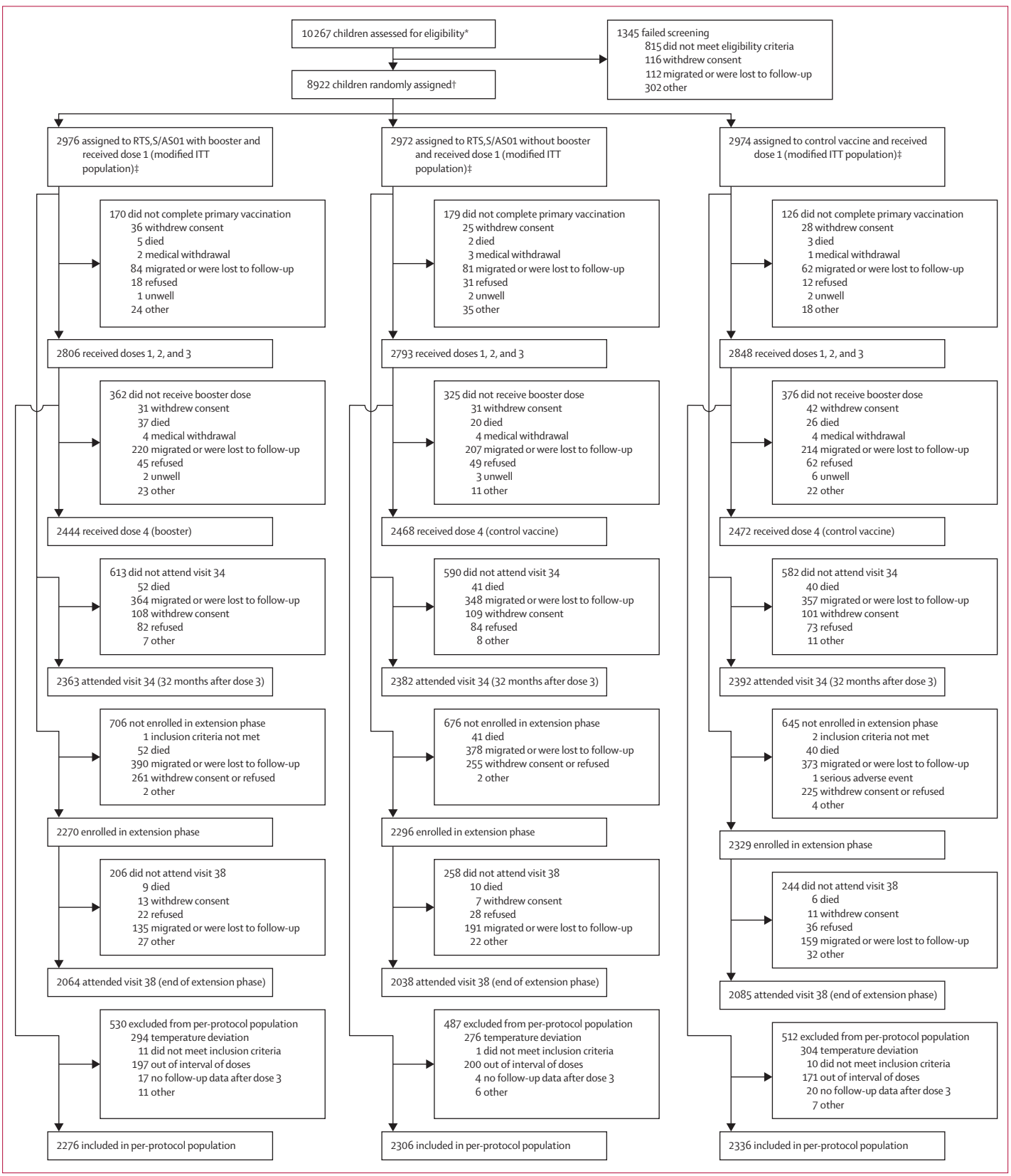
See Online for appendix

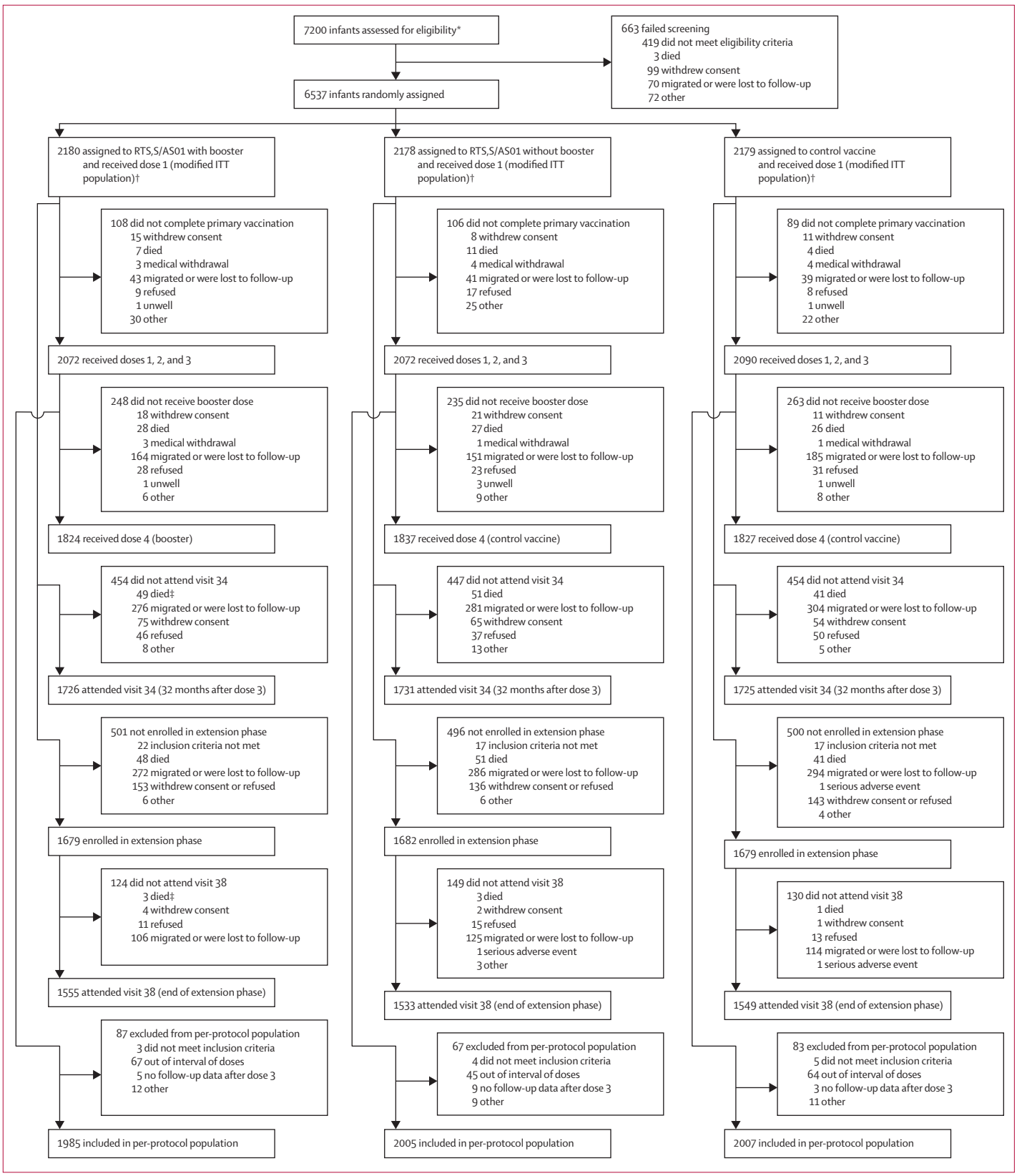
The trial protocol was approved by the ethical review board at each study centre and partner institution and by the national regulatory authority in each country (appendix p 44–45) and the trial was undertaken in accordance with the provisions of the Good Clinical Practice Guidelines.[11]

Randomisation and masking

From March 27, 2009, until Jan 31, 2011, infants aged 6–12 weeks and children aged 5–17 months were recruited and randomly assigned (1:1:1) by block randomisation with minimisation by centre to one of three groups. One group received RTS,S/AS01 at months 0, 1, and 2, followed by a booster dose at month 20 (R3R group); a second group received the RTS,S/AS01 primary vaccination series with meningococcal serogroup C conjugate vaccine (Menjugate, Novartis, Basel, Switzerland) instead of an RTS,S/AS01 booster (R3C group); and the third group received only comparator vaccines: rabies vaccine (Verorab, Sanofi Pasteur, Paris, France) for children and Menjugate for

Figure 1: **Trial profile for participants aged 5–17 months**
Participants' flow in the study in those enrolled in the 5–17 months age category. ITT=intention to treat. *For 70 children, the screening data had not been reported before the database freeze of the previous analyses and these participants were not included in the CONSORT charts published previously. †During monitoring, one participant was found to have been enrolled twice at two different clinics under two different participant numbers. This participant was excluded from the per-protocol analyses. Because of the removal of one participant number from the database, the total number of participants enrolled into the study changed from 15 460 (8923 in the 5–17 months age group), as reported in previous analyses, to 15 459 participants (8922 in the 5–17 months age group) in the final analyses reported here. ‡2867 children in the with booster group, 2887 in the without booster group, and 2905 in the control group received doses 1 and 2.


10 267 children assessed for eligibility*
1345 failed screening
815 did not meet eligibility criteria
116 withdrew consent
112 migrated or were lost to follow-up
302 other
8922 children randomly assigned†
2976 assigned to RTS,S/AS01 with booster and received dose 1 (modified ITT population)‡
2972 assigned to RTS,S/AS01 without booster and received dose 1 (modified ITT population)‡
2974 assigned to control vaccine and received dose 1 (modified ITT population)‡
170 did not complete primary vaccination
36 withdrew consent
5 died
2 medical withdrawal
84 migrated or were lost to follow-up
18 refused
1 unwell
24 other
179 did not complete primary vaccination
25 withdrew consent
2 died
3 medical withdrawal
81 migrated or were lost to follow-up
31 refused
2 unwell
35 other
126 did not complete primary vaccination
28 withdrew consent
3 died
1 medical withdrawal
62 migrated or were lost to follow-up
12 refused
2 unwell
18 other
2806 received doses 1, 2, and 3
2793 received doses 1, 2, and 3
2848 received doses 1, 2, and 3
362 did not receive booster dose
31 withdrew consent
37 died
4 medical withdrawal
220 migrated or were lost to follow-up
45 refused
2 unwell
23 other
325 did not receive booster dose
31 withdrew consent
20 died
4 medical withdrawal
207 migrated or were lost to follow-up
49 refused
3 unwell
11 other
376 did not receive booster dose
42 withdrew consent
26 died
4 medical withdrawal
214 migrated or were lost to follow-up
62 refused
6 unwell
22 other
2444 received dose 4 (booster)
2468 received dose 4 (control vaccine)
2472 received dose 4 (control vaccine)
613 did not attend visit 34
52 died
364 migrated or were lost to follow-up
108 withdrew consent
82 refused
7 other
590 did not attend visit 34
41 died
348 migrated or were lost to follow-up
109 withdrew consent
84 refused
8 other
582 did not attend visit 34
40 died
357 migrated or were lost to follow-up
101 withdrew consent
73 refused
11 other
2363 attended visit 34 (32 months after dose 3)
2382 attended visit 34 (32 months after dose 3)
2392 attended visit 34 (32 months after dose 3)
706 not enrolled in extension phase
1 inclusion criteria not met
52 died
390 migrated or were lost to follow-up
261 withdrew consent or refused
2 other
676 not enrolled in extension phase
41 died
378 migrated or were lost to follow-up
255 withdrew consent or refused
2 other
645 not enrolled in extension phase
2 inclusion criteria not met
40 died
373 migrated or were lost to follow-up
1 serious adverse event
225 withdrew consent or refused
4 other
2270 enrolled in extension phase
2296 enrolled in extension phase
2329 enrolled in extension phase
206 did not attend visit 38
9 died
13 withdrew consent
22 refused
135 migrated or were lost to follow-up
27 other
258 did not attend visit 38
10 died
7 withdrew consent
28 refused
191 migrated or were lost to follow-up
22 other
244 did not attend visit 38
6 died
11 withdrew consent
36 refused
159 migrated or were lost to follow-up
32 other
2064 attended visit 38 (end of extension phase)
2038 attended visit 38 (end of extension phase)
2085 attended visit 38 (end of extension phase)
530 excluded from per-protocol population
294 temperature deviation
11 did not meet inclusion criteria
197 out of interval of doses
17 no follow-up data after dose 3
11 other
487 excluded from per-protocol population
276 temperature deviation
1 did not meet inclusion criteria
200 out of interval of doses
4 no follow-up data after dose 3
6 other
512 excluded from per-protocol population
304 temperature deviation
10 did not meet inclusion criteria
171 out of interval of doses
20 no follow-up data after dose 3
7 other
2276 included in per-protocol population
2306 included in per-protocol population
2336 included in per-protocol population


7200 infants assessed for eligibility*
663 failed screening
419 did not meet eligibility criteria
3 died
99 withdrew consent
70 migrated or were lost to follow-up
72 other
6537 infants randomly assigned
2180 assigned to RTS,S/AS01 with booster and received dose 1 (modified ITT population)†
2178 assigned to RTS,S/AS01 without booster and received dose 1 (modified ITT population)†
2179 assigned to control vaccine and received dose 1 (modified ITT population)†
108 did not complete primary vaccination
15 withdrew consent
7 died
3 medical withdrawal
43 migrated or were lost to follow-up
9 refused
1 unwell
30 other
106 did not complete primary vaccination
8 withdrew consent
11 died
4 medical withdrawal
41 migrated or were lost to follow-up
17 refused
25 other
89 did not complete primary vaccination
11 withdrew consent
4 died
4 medical withdrawal
39 migrated or were lost to follow-up
8 refused
1 unwell
22 other
2072 received doses 1, 2, and 3
2072 received doses 1, 2, and 3
2090 received doses 1, 2, and 3
248 did not receive booster dose
18 withdrew consent
28 died
3 medical withdrawal
164 migrated or were lost to follow-up
28 refused
1 unwell
6 other
235 did not receive booster dose
21 withdrew consent
27 died
1 medical withdrawal
151 migrated or were lost to follow-up
23 refused
3 unwell
9 other
263 did not receive booster dose
11 withdrew consent
26 died
1 medical withdrawal
185 migrated or were lost to follow-up
31 refused
1 unwell
8 other
1824 received dose 4 (booster)
1837 received dose 4 (control vaccine)
1827 received dose 4 (control vaccine)
454 did not attend visit 34
49 died‡
276 migrated or were lost to follow-up
75 withdrew consent
46 refused
8 other
447 did not attend visit 34
51 died
281 migrated or were lost to follow-up
65 withdrew consent
37 refused
13 other
454 did not attend visit 34
41 died
304 migrated or were lost to follow-up
54 withdrew consent
50 refused
5 other
1726 attended visit 34 (32 months after dose 3)
1731 attended visit 34 (32 months after dose 3)
1725 attended visit 34 (32 months after dose 3)
501 not enrolled in extension phase
22 inclusion criteria not met
48 died
272 migrated or were lost to follow-up
153 withdrew consent or refused
6 other
496 not enrolled in extension phase
17 inclusion criteria not met
51 died
286 migrated or were lost to follow-up
136 withdrew consent or refused
6 other
500 not enrolled in extension phase
17 inclusion criteria not met
41 died
294 migrated or were lost to follow-up
1 serious adverse event
143 withdrew consent or refused
4 other
1679 enrolled in extension phase
1682 enrolled in extension phase
1679 enrolled in extension phase
124 did not attend visit 38
3 died‡
4 withdrew consent
11 refused
106 migrated or were lost to follow-up
149 did not attend visit 38
3 died
2 withdrew consent
15 refused
125 migrated or were lost to follow-up
1 serious adverse event
3 other
130 did not attend visit 38
1 died
1 withdrew consent
13 refused
114 migrated or were lost to follow-up
1 serious adverse event
1555 attended visit 38 (end of extension phase)
1533 attended visit 38 (end of extension phase)
1549 attended visit 38 (end of extension phase)
87 excluded from per-protocol population
3 did not meet inclusion criteria
67 out of interval of doses
5 no follow-up data after dose 3
12 other
67 excluded from per-protocol population
4 did not meet inclusion criteria
45 out of interval of doses
9 no follow-up data after dose 3
9 other
83 excluded from per-protocol population
5 did not meet inclusion criteria
64 out of interval of doses
3 no follow-up data after dose 3
11 other
1985 included in per-protocol population
2005 included in per-protocol population
2007 included in per-protocol population

young infants (C3C [control group]; appendix p 19). Young infants received the study vaccine at the same time as the Expanded Program on Immunization vaccines.

Procedures

Participants did not receive malaria treatment before vaccination. The treatment of malaria during the study was done in accordance with national guidelines. Malaria was detected by passive surveillance. The primary case definition for clinical malaria was an illness accompanied by an axillary temperature of at least 37·5°C and *P falciparum* asexual parasitaemia (>5000 parasites per mm³) or a case of malaria meeting the primary case definition of severe malaria according to a predefined algorithm (appendix p 46). The secondary case definition for clinical malaria was illness accompanied by an axillary temperature of at least 37·5°C or reported fever within the past 24 h and *P falciparum* asexual parasitaemia at a density of more than 0 parasites per mm³. The primary case definition for severe malaria was *P falciparum* asexual parasitaemia at a density of more than 5000 parasites per mm³ with one or more markers of severe disease and without diagnosis of a coexisting illness (appendix p 47). The secondary case definition for severe malaria was *P falciparum* asexual parasitaemia at a density of more than 5000 parasites per mm³ with one or more markers of severe disease, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress (defined as lower chest wall indrawing or abnormally deep breathing), a Blantyre coma score of 2 or less (on a scale of 0–5, with higher scores suggesting a higher level of consciousness), two or more observed or reported seizures, hypoglycaemia (glucose less than 2·2 mmol/L), acidosis (base excess ≤–10·0 mmol/L), raised lactate concentration (≥5·0 mmol/L), or haemoglobin concentration of less than 50 g/L.[12] Coexisting illnesses were defined as radiographically proven pneumonia, meningitis established by analysis of cerebrospinal fluid, bacteraemia, or gastroenteritis with severe dehydration.

Fatal malaria was defined as a fatal case meeting the *International statistical classification of diseases and related health problems*, tenth edition, categories B50, B53, and B54. All-cause hospital admission was defined as a medical hospital admission of any cause, excluding planned admissions for medical investigation and care or elective surgery and trauma. All-cause mortality was defined as a fatality of any cause, including mortality in the community and in hospital. Incident severe malaria anaemia was defined as a haemoglobin concentration less than 50 g/L and *P falciparum* asexual parasitaemia at a density of more than 0 parasites per mm³. Malaria hospital admission was defined as a hospital admission accompanied by *P falciparum* parasitaemia at a density greater than 5000 parasites per mm³. Blood transfusion was defined as a child with inpatient admission with documented blood transfusion.

We collected information 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 in the appendix (p 7). Serious AEs (SAEs) were identified during study follow-up by surveillance at health facilities in the study area and through monthly home visits. Verbal autopsies using standardised procedures were done on deaths that occurred outside hospital. SAEs were coded from clinician-assigned diagnoses according to the preferred terms of the *Medical dictionary for regulatory activities*.[13]

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 (appendix pp 7–8). Anti-circumsporozoite 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, 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.[14] Other laboratory and radiological procedures are described in the appendix (pp 9–10).

Outcomes

Analysis of the original coprimary endpoints of the occurrence of malaria up to 12 months after dose 3 and other secondary outcomes have been published previously.[7–9] In this final analysis, we present data for the efficacy of the booster on the occurrence of malaria and other disease endpoints that allow the assessment of the public health effect of the vaccine, such as VE against anaemia, blood transfusion, hospital admission, mortality, and other serious illnesses.

Statistical analysis

All results presented are for the modified intention-to-treat (ITT) population, unless otherwise recorded as per protocol. The modified ITT population included all participants who received at least one dose of vaccine. The per-protocol 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

Figure 2: **Trial profile for participants aged 6–12 weeks**
Participants' flow in the study in those enrolled in the 6–12 weeks age category. ITT=intention to treat. *For 118 infants, the screening data had not been reported before the database freeze of the previous analyses and these participants were not included in the CONSORT charts published previously. †2115 children in the with booster group, 2119 in the without booster group, and 2134 in the control group received doses 1 and 2. ‡For some participants, consent to the extension occurred before visit 34. One participant consented to the extension but died before visit 34. This participant is considered as enrolled into the extension and the reason for not undertaking visit 38 is recorded as died.

	C3C group				R3C group				R3R group				Point estimate of VE unadjusted for covariates R3C *vs* C3C		Point estimate of VE unadjusted for covariates R3R *vs* C3C		Incremental efficacy unadjusted for covariates R3R *vs* R3C	
	N	n	T	n/T	N	n	T	n/T	N	n	T	n/T	VE (95% CI)	p value	VE (95% CI)	p value	VE (95% CI)	p value
5–17 months age category																		
Month 0 to study end	2974	9585	9994·9	0·96	2972	7396	10037·3	0·74	2976	6616	9957·6	0·66	28·3% (23·3 to 32·9)	<0·0001	36·3% (31·8 to 40·5)	<0·0001	..	..
Months 0–32	2974	6768	7088·5	0·95	2972	4711	7180·0	0·66	2976	4078	7099·7	0·57	35·2% (30·5 to 39·5)	<0·0001	43·9% (39·7 to 47·8)	<0·0001	..	..
Months 0–20*	2974	4305	4484·4	0·96	5949	5106	9059·1	0·56	5949	5106	9059·1	0·56	45·1% (41·4 to 48·7)	<0·0001	..	..	..	..
Months 21–32	2700	2442	2609·9	0·94	2717	2076	2621·7	0·79	2679	1592	2601·0	0·61	16·1% (8·5 to 23·0)	<0·0001	37·4% (31·4 to 42·8)	<0·0001	25·6% (18·2 to 32·3)	<0·0001
Month 33 to study end	2309	2817	2912·0	0·97	2267	2685	2861·6	0·94	2236	2539	2862·2	0·89	2·9% (–6·4 to 11·4)	0·53	12·3% (3·6 to 20·1)	0·0062	..	..
Month 21 to study end	2701	5259	5516·3	0·95	2719	4761	5479·1	0·87	2681	4130	5458·9	0·76	11·4% (4·4 to 18·0)	0·0020	25·6% (19·4 to 31·3)	<0·0001	16·2% (9·1 to 22·7)	<0·0001
6–12 weeks age category																		
Month 0 to study end	2179	6170	6147·3	1·00	2178	5444	6174·3	0·88	2180	4993	6156·4	0·81	18·3% (11·7 to 24·4)	<0·0001	25·9% (19·9 to 31·5)	<0·0001	..	..
Months 0–32	2179	4916	5162·4	0·95	2178	4174	5190·7	0·80	2180	3842	5173·3	0·74	20·3% (13·6 to 26·5)	<0·0001	27·8% (21·7 to 33·4)	<0·0001	..	..
Months 0–20*	2179	2751	3273·6	0·84	4358	4252	6583·6	0·65	4358	4252	6583·6	0·65	27·0% (21·1 to 32·5)	<0·0001	..	..	..	..
Months 21–32	1976	2156	1889·3	1·14	1995	2079	1893·0	1·10	1966	1671	1888·4	0·88	7·6% (–1·4 to 15·9)	0·096	28·1% (20·6 to 34·8)	<0·0001	22·3% (14·0 to 29·8)	<0·0001
Month 33 to study end	1657	1254	986·1	1·27	1658	1271	984·0	1·29	1654	1154	984·9	1·17	4·4% (–6·7 to 14·3)	0·42	10·5% (0·2 to 19·7)	0·046	..	..
Month 21 to study end	1976	3410	2874·2	1·19	1996	3349	2876·7	1·16	1966	2822	2871·5	0·98	7·6% (–0·8 to 15·3)	0·076	23·5% (16·4 to 30·1)	<0·0001	17·5% (9·5 to 24·8)	<0·0001

Analyses were by modified intention to treat. p values were calculated using negative binomial regression. C3C=control group. N=number of participants. n=number of episodes meeting the case definition. n/T=incidence. R3C=RTS,S/AS01 primary schedule without booster. R3R=RTS,S/AS01 primary schedule with booster. T=person-years at risk. VE=vaccine efficacy (negative binomial model). *Data from a previous analysis that compared R3R plus R3C with C3C.

Table 1: **Efficacy against clinical malaria (primary case definition) of a primary schedule with or without a booster dose and incremental efficacy of the booster dose**

between episodes within the same participant. Overall estimates were adjusted for study site as a fixed effect, whereas site estimates were unadjusted for covariates. Inter-site variation was assessed by site interaction terms. VE over time was assessed by calculating VE during consecutive time periods, months 0–20, months 21–32, and month 33 to study end. The incremental efficacy of the RTS,S/AS01 booster dose was calculated for the time period after month 20, 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 endpoints (ie, severe malaria, malaria hospital admission, fatal malaria, all-cause hospital admission, all-cause mortality, incident severe anaemia, and blood transfusion) was estimated as a relative risk reduction with Fisher's exact p values. The number of cases averted over

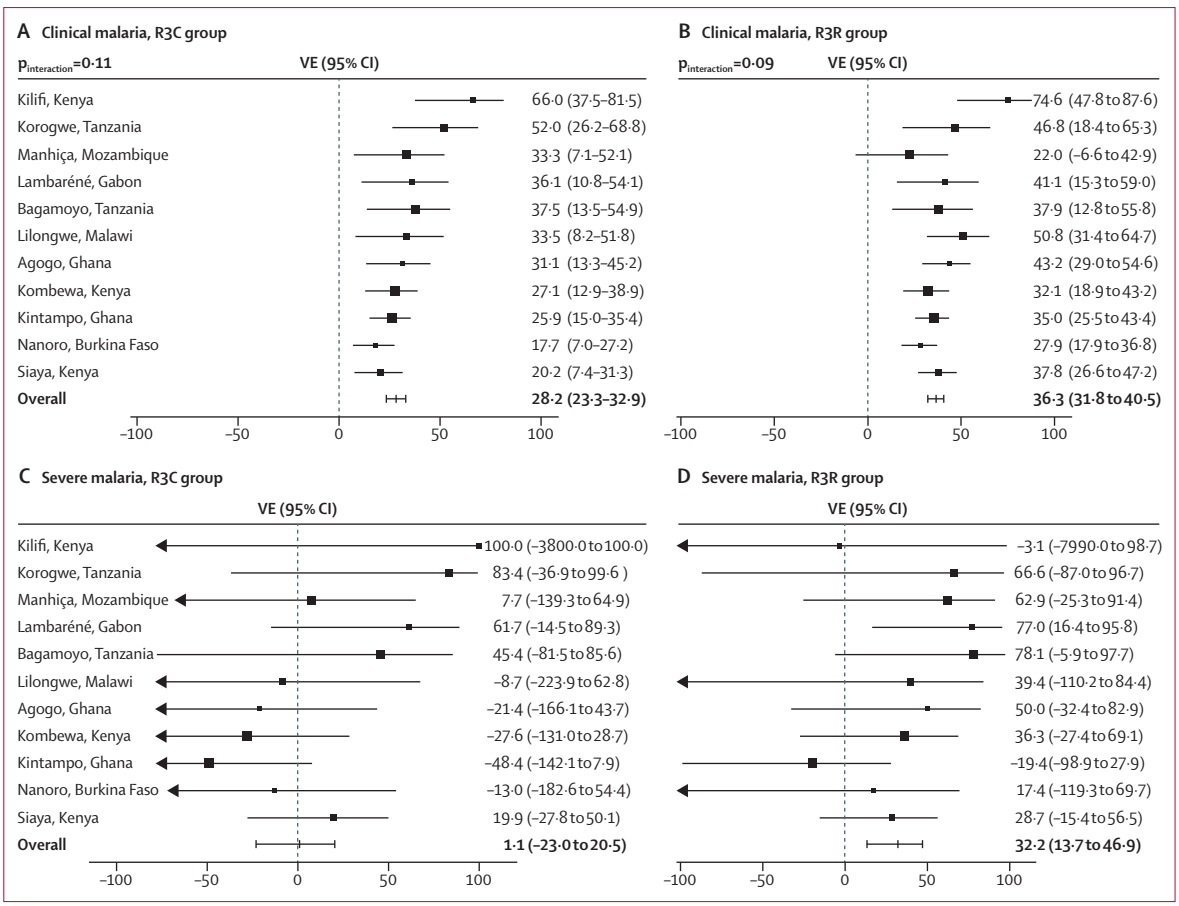


Figure 3: **Vaccine efficacy against clinical and severe malaria by study site in the 5–17 months age category**
VE against all episodes of clinical malaria (primary case definition) in (A) the R3C group and (B) the R3R group from month 0 to study end; and VE against severe malaria (primary case definition) in (C) the R3C group and (D) the R3R group from month 0 to study end. Study sites are ordered from lowest (Kilifi) to highest (Siaya) incidence of clinical malaria (secondary case definition) measured in control infants 6–12 weeks of age at enrolment during 12 months of follow-up. $p_{interaction}$ not calculated for (C) or (D). Analyses were by modified intention to treat. Bars are 95% CIs. The size of each square is proportional to the number of participants enrolled at each study site. The following numbers of children aged 5–17 months were enrolled by site for all three groups (R3R, R3C, and C3C) together: 600 in Kilifi, 912 in Korogwe, 1002 in Manhiça, 704 in Lambaréné, 903 in Bagamoyo, 800 in Lilongwe, 600 in Agogo, 1000 in Kombewa, 1002 in Kintampo, 600 in Nanoro, and 799 in Siaya. R3C=RTS,S/AS01 primary schedule without booster. C3C=control group. R3R=RTS,S/AS01 primary schedule with booster. VE=vaccine efficacy.

time was calculated as the sum of 3-monthly differences in the estimated number of cases between the control and the RTS,S/AS01 groups (R3R and R3C combined up to the time of booster dose and R3R and R3C separately after the booster dose) and expressed per 1000 participants vaccinated. 14 days after an episode were subtracted from the time at risk and no malaria events were counted during this period. 95% CIs were estimated by bootstrapping, using the 2·5 and 97·5 centiles of 1000 replicates obtained by sampling participants, stratified by site.[15] The primary case definition of clinical malaria was used for calculation of VE, whereas a more sensitive secondary case definition was used for the assessment of the number of cases averted of 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. p values less than 0·05 were deemed significant.

This trial is registered with ClinicalTrials.gov, number NCT00866619.

Role of the funding source

GSK Biologicals SA were involved in study design, and coordinated data collection, data analysis, data interpretation, and writing of the report. The PATH Malaria Vaccine Initiative (MVI) contributed to study design and data interpretation, but were not involved in data collection, data analysis, or writing of the report. The RTS,S Clinical Trials Partnership had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

8922 children and 6537 young infants were enrolled and included in the modified ITT analyses; of these, 6918 (78%) children and 5997 (92%) young infants were included in the per-protocol analyses (figures 1 and 2).

	C3C group			R3C group			R3R group			Point estimate of VE unadjusted for covariates R3C *vs* C3C		Point estimate of VE unadjusted for covariates R3R *vs* C3C	
	N	n	Proportion affected*	N	n	Proportion affected*	N	n	Proportion affected*	VE (95% CI)	p value	VE (95% CI)	p value
5–17 months age category													
Month 0 to study end	2974	171	0·06	2972	169	0·06	2976	116	0·04	1·1% (–23·0 to 20·5)	0·96	32·2% (13·7 to 46·9)	0·0009
Months 0–32	2974	152	0·05	2972	145	0·05	2976	99	0·03	4·5% (–20·6 to 24·5)	0·72	34·9% (15·6 to 50·0)	0·0006
Months 0–20†	2974	118	0·04	5949	156	0·03	5949	156	0·03	33·9% (15·3 to 48·3)	0·0007	..	..
Months 21–32	2701	42	0·02	2717	61	0·02	2679	43	0·02	–44·4% (–119·0 to 4·1)	0·073	–3·2% (–61·8 to 34·1)	0·91
Month 33 to study end	2309	20	0·01	2267	31	0·01	2236	23	0·01	–57·9% (–192·0 to 12·8)	0·12	–18·8% (–128·0 to 37·6)	0·65
Month 21 to study end	2702	62	0·02	2719	88	0·03	2681	64	0·02	–41·0% (–98·5 to –0·8)	0·038	–4·0% (–50·0 to 27·8)	0·86
6–12 weeks age category													
Month 0 to study end	2179	116	0·05	2178	104	0·05	2180	96	0·04	10·3% (–17·9 to 31·8)	0·45	17·3% (–9·4 to 37·5)	0·16
Months 0–32	2179	101	0·05	2178	93	0·04	2180	89	0·04	7·9% (–23·3 to 31·2)	0·61	11·9% (–18·3 to 34·5)	0·37
Months 0–20†	2179	66	0·03	4358	121	0·03	4358	121	0·03	8·3% (–25·7 to 32·6)	0·58	..	..
Months 21–32	1976	43	0·02	1995	40	0·02	1966	29	0·01	7·9% (–45·1 to 41·6)	0·74	32·2% (–11·1 to 59·2)	0·12
Month 33 to study end	1657	16	0·01	1658	14	0·01	1654	12	0·01	12·6% (–91·2 to 60·5)	0·72	24·9% (–69·3 to 67·6)	0·57
Month 21 to study end	1976	58	0·03	1996	52	0·03	1966	39	0·02	11·2% (–31·3 to 40·2)	0·56	32·4% (–3·2 to 56·2)	0·064

Analyses were by modified intention to treat. p values were calculated using a two-sided Fisher's exact test. C3C=control group. N=number of participants. n=number of participants with at least one event in each group. R3C=RTS,S/AS01 primary schedule without booster. R3R=RTS,S/AS01 primary schedule with booster. VE=vaccine efficacy (1–relative risk for severe malaria). *Proportion of participants who reported at least one event. †Data from a previous analysis that compared R3R plus R3C with C3C.

Table 2: **Efficacy against severe malaria (primary case definition) of a primary schedule with or without a booster dose**

The median follow-up after dose 1 in the 6–12 weeks age group was 37·8 months (IQR 34·3–41·0) in R3R, 37·7 months (34·1–41·1) in R3C, and 37·8 months (34·1–41·1) in C3C (median overall 37·7 months, (IQR 34·1–41·1; modified ITT). The median follow-up after dose 1 in the 5–17 months age group was 48·1 months (IQR 36·7–50·1) in R3R, 48·1 months (37·9–50·1) in R3C, and 48·4 months (41·3–50·1) in C3C (median overall 48·2 months, IQR 39·4–50·1; modified ITT). Baseline characteristics were similar in the three study groups in each age category and between the modified ITT and per-protocol populations, but differed between sites (appendix p 20). Insecticide-treated bednet use was consistently high, although it varied between study sites (appendix p 25). 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; appendix p 48). Artemisinin combination therapy was the first-line antimalarial and was given to treat 99% of malaria cases (42 353 of 42 977 episodes in children and 29 012 of 29 257 episodes in infants; appendix p 25). 158 of 8922 (1·8%, 95% CI 1·5–2·1) children and 148 of 6537 (2·3%, 1·9–2·7) young infants died during follow-up (month 0 to study end; appendix pp 49, 59).

In the modified ITT population, from month 0 until study end, compared with 9585 episodes of clinical malaria that met the primary case definition in children in the C3C group, 6616 episodes occurred in children in the R3R group (VE 36·3%, 95% CI 31·8–40·5) and 7396 in the R3C group (28·3%, 23·3–32·9; table 1; appendix pp 28, 69). Corresponding data in the per-protocol population were 8352 episodes in the C3C group compared with 5691 in the R3R group (VE 39·0%, 95% CI 34·3–43·3) and 6597 in the R3C group (26·2%, 20·8–31·2; appendix p 67). Efficacy was similar in children vaccinated when 5–11 months of age or 12–17 months of age (appendix p 71). Efficacy varied by site with or without booster vaccination, but these differences were not significant ($p_{interaction}$=0·09 and $p_{interaction}$=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 month 33 to study end 2·9%, 95% CI –6·4 to 11·4; table 1; appendix p 29). By contrast, VE persisted to study end in the R3R group (VE month 33 to study end 12·3%, 95% CI 3·6–20·1; table 1 and appendix p 29). The incremental efficacy provided by the booster dose during the 12 months after booster vaccination was 25·6% (95% CI 18·2–32·3; table 1 and appendix p 30).

In the modified ITT population, from month 0 until study end, compared with 171 children in the C3C group, 116 children in the R3R group (VE 32·2%, 95% CI 13·7 to 46·9) and 169 in the R3C group (1·1%, –23·0 to 20·5) experienced at least one episode of severe malaria that met the primary case definition (table 2; appendix p 69). Corresponding data in the per-protocol population were 135 children affected in the C3C group compared with 94 in the R3R group (VE 28·5%, 95% CI 6·3 to 45·7) and 141 in the R3C group (–5·8%, –35·0 to 17·0; appendix p 67). From month 0 until month 20, 156 children in the combined R3R plus R3C group experienced severe malaria compared with 118 in the C3C group (VE 33·9%, 95% CI 15·3–48·3); however, from month 21 to study

end, compared with 62 children in the C3C group, 64 in the R3R group (VE –4·0%, 95% CI –50·0 to 27·8) and 88 in the R3C group (–41·0%, –98·5 to –0·8; table 2) experienced severe disease. Figures 3C and D show VE against severe malaria by site over the study period (month 0 to study end); although not significant, there is a pattern for the higher point estimates of VE to occur in the lower transmission settings as is the case for VE against clinical malaria and negative point estimates (with large CIs) to occur in the R3C high transmission areas. The appendix (p 31) shows the distribution of markers of severe malaria in the three study groups; illnesses characterised by a low coma score seemed to occur more frequently in children who had received RTS,S/AS01, but confidence intervals overlap. 598 (97%) of 618 children and 409 (98%) of 418 young infants admitted with severe malaria recovered without major lasting sequelae (appendix pp 72–73).

From month 0 until study end, compared with 44 children in the C3C group, 23 children in the R3R group (VE 47·8%, 95% CI 11·6 to 69·9) and 34 in the R3C group (22·7%, –23·8 to 52·1) had at least one episode of incident severe malaria anaemia; and compared with 347 children in the C3C group, 227 children in the R3R group (34·6%, 22·5 to 44·9) and 286 in the R3C group (17·5%, 3·3 to 29·7) were admitted to hospital for malaria at least once (appendix p 74–76). Compared with 771 children in the C3C group, 644 children in the R3R group (VE 16·5%, 7·2–24·9) and 682 in the R3C group (11·5%, 1·7–20·3) were admitted to hospital at least once for any cause (appendix p 76). Between month 0 and study end, compared with 109 controls, 78 children in the R3R group (VE 28·5%, 95% CI 3·5 to 47·2) and 91 in the R3C group (16·5%, –11·4 to 37·5) had at least one blood transfusion (appendix p 76).

Significant efficacy against prevalent parasitaemia was noted in the R3R group compared with the C3C group at the cross-sectional assessments at month 32 (p<0·0001), month 44 (p=0·019), and study end (early p=0·018 and late p=0·044), and in the R3C group at month 32 (p=0·0001), but not at study end (appendix p 78). No significant VE was noted against incident bacteraemia, pneumonia, all-cause mortality, or malaria mortality (appendix pp 74–76) and there was no effect on indices of malnutrition with or without a booster dose (appendix p 80).

From month 0 until study end, 1774 cases of clinical malaria per 1000 children (95% CI 1387–2186; range across sites 205–6565) were averted in the R3R group and 1363 per 1000 children (95% CI 995–1797; range 215–4443) in the R3C group (table 3; figure 4; appendix p 83). The numbers of cases averted per 1000 children in the R3R group and R3C group, respectively, were 19 (95% CI 4 to 35) and eight (–9 to 26) for severe malaria, 40 (19 to 64) and 26 (4 to 51) for malaria hospital admission, 59 (18 to 103) and 41 (0 to 84) for all-cause hospital admission, 11 (1 to 24) and nine (–3 to 21) for severe anaemia, and 15 (1 to 31) and 13 (–1 to 28) for blood transfusions (table 3). The number of cases of clinical and severe malaria averted varied substantially by study site; the highest numbers of cases of clinical malaria averted were noted in areas of high malaria incidence, such as Siaya, Kenya, and Nanoro, Burkina Faso (figure 4A).

	5–17 months age category		6–12 weeks age category	
	R3C group	R3R group	R3C group	R3R group
Clinical malaria (secondary case definition)*				
Months 0–20	963 (807 to 1133)†	..	518 (341 to 687)†	..
Months 0–32	1221 (973 to 1483)	1475 (1234 to 1733)	526 (200 to 819)	873 (573 to 1158)
Month 0 to study end‡	1363 (995 to 1797)	1774 (1387 to 2186)	558 (158 to 926)	983 (592 to 1337)
Malaria hospital admission				
Months 0–20	42 (28 to 59)†	..	8 (–9 to 25)†	..
Months 0–32	32 (13 to 53)	44 (26 to 64)	5 (–17 to 27)	14 (–10 to 35)
Month 0 to study end‡	26 (4 to 51)	40 (19 to 64)	14 (–13 to 39)	18 (–8 to 42)
Severe malaria (secondary case definition)				
Months 0–20*	19 (8 to 32)†	..	5 (–8 to 18)†	..
Months 0–32	12 (–2 to 27)	20 (7 to 34)	5 (–13 to 24)	9 (–8 to 28)
Month 0 to study end‡	8 (–9 to 26)	19 (4 to 35)	8 (–13 to 28)	12 (–6 to 32)
Fatal malaria				
Months 0–20*	0 (–2 to 3)†	..	–1 (–3 to 2)†	..
Months 0–32	–1 (–4 to 3)	1 (–2 to 4)	–1 (–5 to 2)	–2 (–5 to 2)
Month 0 to study end‡	–2 (–7 to 2)	1 (–3 to 5)	–3 (–7 to 1)	–2 (–6 to 2)
All-cause hospital admission				
Months 0–20*	57 (29 to 88)†	..	22 (–16 to 60)†	..
Months 0–32	53 (16 to 92)	64 (29 to 103)	15 (–32 to 70)	38 (–9 to 89)
Month 0 to study end‡	41 (0 to 84)	59 (18 to 103)	24 (–27 to 82)	36 (–17 to 90)
All-cause mortality				
Months 0–20*	–1 (–7 to 4)†	..	–5 (–12 to 3)†	..
Months 0–32	–3 (–10 to 3)	0 (–7 to 6)	–5 (–13 to 3)	–6 (–14 to 3)
Month 0 to study end‡	–5 (–12 to 3)	–1 (–9 to 6)	–5 (–14 to 4)	–6 (–15 to 3)
Incident severe anaemia				
Months 0–20*	8 (1 to 15)†	..	0 (–8 to 11)†	..
Months 0–32	9 (0 to 19)	10 (1 to 20)	0 (–13 to 15)	3 (–9 to 16)
Month 0 to study end‡	9 (–3 to 21)	11 (1 to 24)	–1 (–16 to 15)	3 (–11 to 17)
Blood transfusion				
Months 0–20*	13 (4 to 21)†	..	1 (–10 to 13)†	..
Months 0–32	13 (1 to 24)	15 (3 to 27)	2 (–15 to 20)	5 (–9 to 23)
Month 0 to study end	13 (–1 to 28)	15 (1 to 31)	1 (–18 to 19)	4 (–12 to 23)

Data are number of cases averted per 1000 participants (95% CI). Analyses were by modified intention to treat. R3C=RTS,S/AS01 primary schedule without booster. R3R=RTS,S/AS01 primary schedule with booster. *This definition was used for this analysis because, during routine clinical practice, these children would normally receive a full course of anti-malarial treatment. †The schedule without a booster (R3C) and the schedule with a booster (R3R) were pooled (R3R plus R3C) to calculate the number of cases averted. ‡Median of 48 months after the first dose for the 5–17 months age category and 39 months after the first dose for the 6–12 weeks age category.

Table 3: **Number of cases averted per 1000 participants in children or young infants immunised with a primary vaccination schedule with or without a booster dose**

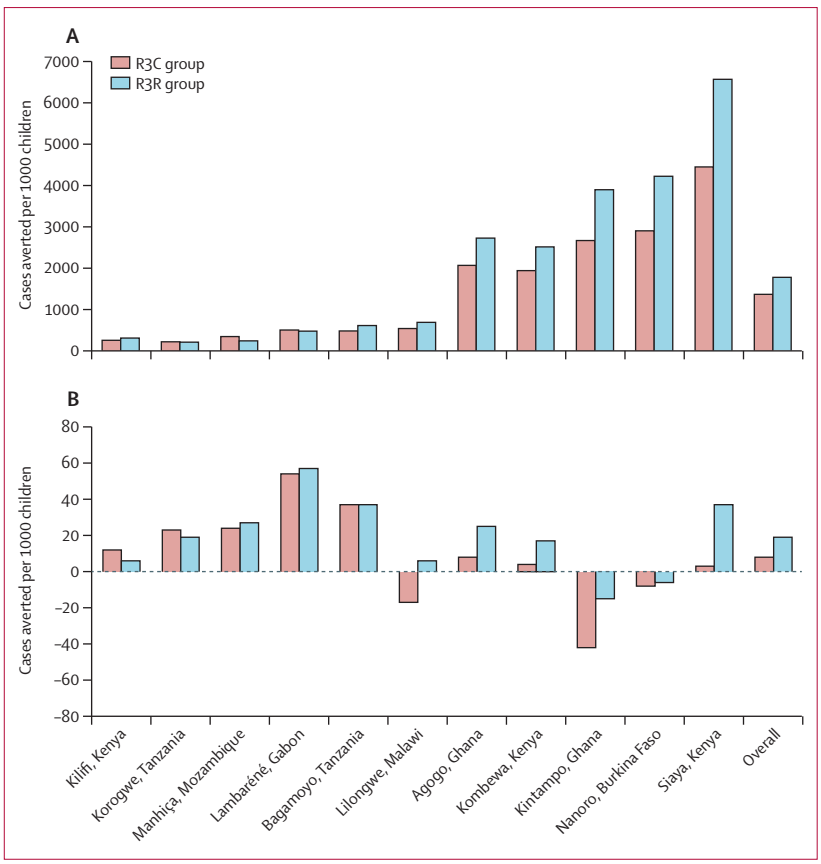


Figure 4: **Cases of clinical and severe malaria averted at each site during 48 months of follow-up in the 5–17 months age category**

Analyses were by modified intention to treat. Data are ordered by increasing malaria incidence at each study site. Cases averted of (A) clinical malaria (secondary case definition) and (B) severe malaria (secondary case definition) between month 0 and study end. The secondary case definition of severe malaria was used for this analysis because, during routine clinical practice, these children would normally receive a full course of antimalarial treatment. R3C=RTS,S/AS01 primary schedule without booster. R3R=RTS,S/AS01 primary schedule with booster.

In the modified ITT population, from month 0 until study end, compared with 6170 episodes of clinical malaria that met the primary case definition in young infants in the C3C group, 4993 episodes occurred in the R3R group (VE 25·9%, 95% CI 19·9–31·5) and 5444 in the R3C group (18·3%, 11·7–24·4; table 1; appendix pp 33, 86). Corresponding data in the per-protocol population were 5666 episodes in the C3C group compared with 4532 in the R3R group (VE 26·7%, 95% CI 20·5–32·4) and 5072 in the R3C group (18·2%, 11·4–24·5; appendix p 84). Estimates of VE 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, compared with the C3C group, was still present in the R3R group during the last study period (VE month 33 to study end 10·5%, 95% CI 0·2 to 19·7), but not in the R3C group (4·4%, –6·7 to 14·3; table 1; appendix p 34). The incremental efficacy against clinical malaria provided by the booster dose during the 12 months after booster vaccination was 22·3% (95% CI 14·0–29·8; table 1; appendix p 35).

In the modified ITT population, from month 0 to study end, compared with 116 young infants in the C3C group, 96 young infants in the R3R group (VE 17·3%, 95% CI –9·4 to 37·5) and 104 in the R3C group (10·3%, –17·9 to 31·8) experienced at least one episode of severe malaria that met the primary case definition (table 2). Compared with 35 young infants in the C3C group, 24 young infants in the R3R group (VE 31·5%, 95% CI –18·5 to 61·0) and 31 in the R3C group (11·4%, –47·9 to 47·2) had at least one episode of incident severe malaria anaemia (appendix pp 88). Compared with 188 young infants in the C3C group, 142 young infants in the R3R group (VE 24·5%, 95% CI 5·6 to 39·7) and 167 in the R3C group (11·1%, –10·1 to 28·3) were admitted to hospital at least once for malaria (appendix p 88). No protection was noted against all-cause hospital admission, bacteraemia, pneumonia, all-cause mortality, or malaria mortality (appendix p 88–90). VE did not differ between the control and RTS,S/AS01 vaccine with or without a booster groups in terms of prevalent parasitaemia or indices of malnutrition (appendix p 92–93).

Between month 0 and study end, the number of cases averted per 1000 young infants in the R3R and R3C study groups, respectively, were 983 (95% CI 592–1337; range across sites –30 to 3406) and 558 (95% CI 158–926; range –172 to 2178) for clinical malaria, 12 (–6 to 32) and eight (–13 to 28) for severe malaria, 18 (–8 to 42) and 14 (–13 to 39) for malaria hospital admissions, and 36 (–17 to 90) and 24 (–27 to 82) for all-cause hospital admissions (figure 6; table 3; appendix p 94).

Anti-circumsporozoite antibody responses are shown in the appendix (p 36). 1 month after the booster dose with RTS,S/AS01, the geometric mean titre in children in the R3R group was 318·2 EU/mL (95% CI 295·1–343·0) compared with 34·2 EU/mL (30·5–38·3) in the R3C group (per-protocol population; appendix p 95). The comparable data in young infants were 169·9 EU/mL (95% CI 153·8–187·7) and 6·2 EU/mL (5·4–7·0), respectively (per-protocol population; appendix p 96). 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) in the R3R group and 19·3 EU/mL (17·2–21·8) in the R3C group in children and 15·9 EU/mL (13·8–18·3) in the R3R group and 3·7 EU/mL (3·3–4·2) in the R3C group in young infants (appendix pp 36, 95–96). Anti-circumsporozoite antibodies were categorised by tertile. Infants who were RTS,S/AS01 vaccine recipients and whose antibody response was in the top tertile 1 month after primary vaccination series had a 36·9% (95% CI 17·3–51·8; p=0·0009) reduction in risk of subsequent malaria episodes compared with those in the lowest tertile. No significant risk reduction was noted in children in the highest tertile compared with those in the lowest tertile (3·6% reduction, 95% CI –25·6 to 26·0; p=0·79; appendix pp 37–39).

The RTS,S/AS01 booster dose was more reactogenic than the comparator vaccine in both children and young

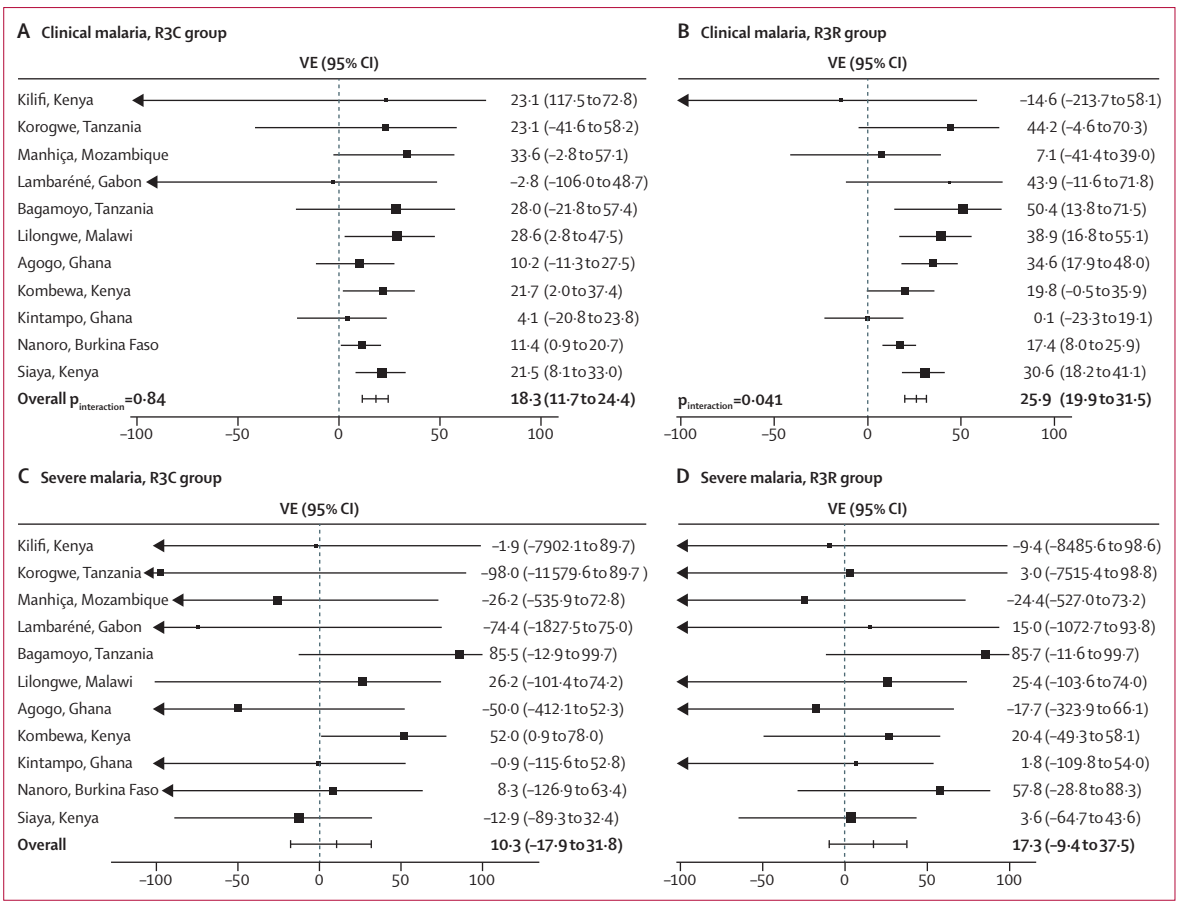


Figure 5: **Vaccine efficacy against clinical and severe malaria by study site in the 6–12 weeks age category**
VE against all episodes of clinical malaria (primary case definition) in (A) the R3C group and (B) the R3R group from month 0 to study end; and VE against severe malaria (primary case definition) in (C) the R3C group and (D) the R3R group from month 0 to study end. Study sites are ordered from lowest (Kilifi) to highest (Siaya) modified incidence of clinical malaria (secondary case definition) measured in control infants 6–12 weeks of age at enrolment during 12 months of follow-up. Analyses were by modified intention to treat. Bars are 95% CIs. The size of each square is proportional to the number of participants enrolled at each study site. The following numbers of infants aged 6–12 weeks were enrolled by site for all three groups (R3R, R3C, and C3C) together: 304 in Kilifi, 593 in Korogwe, 635 in Manhiça, 226 in Lambaréné, 802 in Bagamoyo, 826 in Lilongwe, 688 in Agogo, 631 in Kombewa, 331 in Kintampo, 681 in Nanoro, and 820 in Siaya. C3C=control group. R3C=RTS,S/AS01 primary schedule without booster. R3R=RTS,S/AS01 primary schedule with booster. VE=vaccine efficacy.

infants, with a higher frequency of both systemic and local reactions within 7 days of vaccination in the R3R group than in the R3C or C3C groups (appendix p 97–98). However, grade 3 reactions were rare, except for grade 3 fevers (>39°C), which occurred in 34 of 641 children (5·3%, 95% CI 3·7–7·3) and nine of 608 infants (1·5%, 0·7–2·8) after a booster dose of RTS,S/AS01 (appendix pp 41, 97–98). The incidence of generalised convulsive seizures within 7 days of a booster dose in children was 2·5 per 1000 doses in the R3R group, 1·2 per 1000 doses in the R3C group, and 0·4 per 1000 doses in the C3C group, and in young infants it was 2·2 per 1000 doses in the R3R group, 0·0 per 1000 doses in the R3C group, and 0·5 per 1000 doses in the C3C group (appendix p 99).

The incidence of SAEs overall and of unsolicited AEs within 30 days of booster vaccination were similar in all three study groups (appendix pp 49, 59, 100–103). Meningitis was reported as a SAE in 22 children: 11 in the R3R group, ten in the R3C group, and one in the C3C group (appendix pp 49, 59). Five of these 22 cases were reported after booster vaccination: two in the R3R group and three in the R3C group. A bacterial cause was identified in ten cases (five meningococcal, three *Haemophilus influenzae*, one pneumococcal, and one tuberculosis), all in children in the R3R or R3C groups. Meningitis was reported as a SAE in 18 young infants; no imbalance in cases of meningitis was noted in the younger age group (five cases in the R3R group, seven in the R3C group, and six in the C3C group). Meningitis cases were not temporally related to vaccination (appendix p 42). Most study sites reported one to three cases of meningitis, but 15 cases were reported from Lilongwe, Malawi, and five from Kombewa, Kenya. All cases of meningitis and other infections of the nervous system reported as a 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 and six in R3C), 11 as not

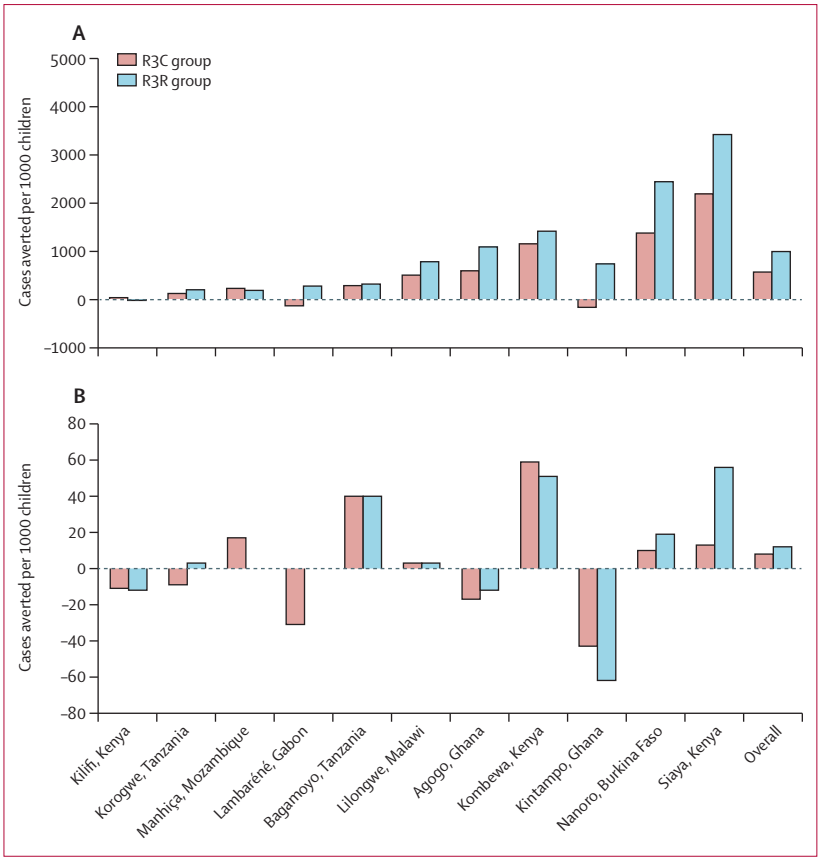


Figure 6: **Cases of clinical and severe malaria averted at each site during 38 months of follow-up in the 6–12 weeks age category**

Analyses were by modified intention to treat. Data are ordered by increasing malaria incidence at each study site. Cases averted of (A) clinical malaria (secondary case definition) and (B) severe malaria (secondary case definition) between month 0 and study end. The secondary case definition of severe malaria was used for this analysis because, during routine clinical practice, these children would normally receive a full course of antimalarial treatment. R3C=RTS,S/AS01 primary schedule without booster. R3R=RTS,S/AS01 primary schedule with booster.

a meningitis case (five in R3R, four in R3C, and two in C3C), and ten 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, and 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 and one in R3C).

Discussion

The decrease in efficacy of RTS,S/AS01 against clinical and severe malaria over time since vaccination, which was 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, VE against clinical malaria during the full follow-up period in the absence of a booster dose were about 28% in children and 18% in young infants, which resulted in a substantial reduction in the number of cases of clinical malaria. Protection against clinical malaria was prolonged in both children and young infants by a booster dose, which increased the number of cases prevented across sites in both children and 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 noted during the study period in young infants or children who did not receive a booster dose of vaccine. This finding contrasts with our earlier report of a significant reduction in severe malaria in children in the older age category who were followed up until the time of booster vaccination.[8,9] This anomaly can be 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 compared with children in the control group, whereas this was not noted in children who received a booster dose of vaccine. This increased risk occurred 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 finding might have occurred by chance; the number of cases was low and a similar pattern was not noted for cases of uncomplicated malaria. However, vaccination, by providing protection against malaria infection, might have 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 ongoing 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 consideration should be given to inclusion of a booster dose, especially in higher transmission areas, and the possible effect of administration of further booster doses will need to be explored.

Vaccination with RTS,S/AS01 significantly reduced overall hospital admissions, admissions because of 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. The numbers of cases averted of these endpoints were lower in infants vaccinated at age 6–12 weeks. No significant effect on overall mortality, malaria mortality, pneumonia, or sepsis was noted in either age category. The latter finding is surprising because malaria seems to be an important risk factor for invasive bacterial infections.[16] Failure to detect an effect of RTS,S/AS01 on these secondary outcomes, including mortality, might have been caused in part by the high level of clinical care provided during the trial, including high coverage with

insecticide-treated bednets and enhanced access to effective treatment of malaria and other disorders. RTS,S/AS01 might have reduced the numbers of cases averted of these endpoints even more 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-circumsporozoite geometric mean titres in both young infants and children, as noted previously in adults immunised with an earlier formulation of the vaccine (RTS,S/AS02),[17] but the anti-circumsporozoite geometric mean titres after the booster remained lower than concentrations after the primary course and the booster effect was only transitory. Changes in anti-circumsporozoite 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 we cannot conclude definitively that children who received the booster dose had acquired immunological memory. However, limited data from a previous study[18] suggest that the antibody response to a booster dose in children who had been primed with RTS,S/AS01 was greater than that reported in participants who had received a single dose without priming, and that some immunological memory had been induced with the prime series of RTS,S/AS01 vaccination. 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.

SAEs were reported in about a quarter of children in the trial, with a similar incidence in all study groups, but only 0·3% were judged to be vaccine related (appendix pp 49, 59). 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,[9] remained. Five new cases of meningitis were recorded from month 21 until the end of the trial in children in the RTS,S/AS01 groups, but none occurred 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 noted in young infants. This imbalance in cases of meningitis in children could be a chance finding because comparisons were made across groups for many different diagnostic classifications of SAEs, most of the cases were clustered in two sites, and there was no temporal relation to vaccination. If children who received RTS,S/AS01 do have an increased risk of meningitis, the mechanism that could have brought this about is difficult to understand. If RTS,S/AS01 is licensed, post-registration studies will be done to establish the significance of this finding. The incidence of fever in the 1 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 were accompanied by generalised convulsive seizures.

Despite its large size and attention to detail, this trial has some weaknesses. The per-protocol population was high in young infants but lower in children because of a loss of data from one centre after administration of vaccine affected by a temperature deviation. At one centre (Bagamoyo, Tanzania), there was a concern about the quality of the work of two field workers assigned to undertake monthly home visits, but further investigation found no evidence that this had led to under-reporting of SAEs. Differences in the effect of RTS,S/AS01 were found between study sites, but because of the infrequency of assessment of some of the trial endpoints, including severe malaria, site-to-site comparisons should be made with caution. The detailed study analyses done at several timepoints 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 might have limited the ability of the trial to detect an effect on mortality or other severe outcomes.

An application for a Committee for Medicinal Products for Human Use (CHMP) scientific opinion on RTS,S/AS01 through the European Medicines Agency Article 58 procedure is under review. If a positive scientific opinion is obtained from the CHMP and the vaccine is prequalified by WHO, malaria-endemic countries will need to decide whether to license and use 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[19] 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 and the Strategic Advisory Group of Experts committees, which will formulate WHO's recommendations on the use of RTS,S/AS01. The results provided in 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 used correctly, has the potential to prevent millions of cases of malaria.

Contributors

SAb, TA, STA, DA, JJA, KPA, WRB, PBe, UD'A, SGe, BG, MJH, IH, SKar, PGK, TL, DLa, AL, BL, MLe, MLi, JL, EMa, KM, FM, PN, OO-A, AOlo, LO, WO, SO-A, JSa, BS, LS, MT, HT, and JV designed the study. OA, SAb, BPA, AAA, EA, SAd, TA, STA, SAh, PAi, PAk, AA, PAl, JA, DA, IA, KPA, NA, KA, EB, AB-P, TB, PBe, OB, PBu, HB, DC, CC, RC, JCow, KD, DD, CD, AE, JFF, SGe, JG, SGo, CG, AH, MJH, SI, EJ, WK, ALK, SKaf, PK, SKar, KK, CK, SKh, FK, VM-K, AK, PGK, KFL, BL, MLe, EL, PL, JL, DCM, EMa, LM, MM, CMah, CMai, AMa, SMan, KM, FM, MML, SMas, JM, AMd, RM, BMm, AMo, BMo, AMt, TM, SMw, EMz, PN, RN, JOc, CO, SOd, BO, GO, RO, AOlo, JOm, MO, AOt, KO, LO, WO, NO, FO, JBO, SOu, HOB, SO-A, JOy, VP, TR, JSa, NS, DSa, TS, HS, JSy, MCT, GT, TT, TGT, HT, BT, IV, EV, AW, AKY, and ZY collected data. AMo, JJA, DH, and MLi developed the analysis plan for the data. JJA, DH, and MLi vouch for the data and analysis. TA, STA,

PAi, PAk, UD'A, DA, JJA, JCoh, SGe, BG, YG, MJH, DH, IH, PK, SKar, DK, PGK, DLa, AL, DLe, MLi, JL, AMt, PN, BO, AOli, LO, WO, SO-A, JSa, NS, BS, DSc, LS, HT, and JV interpreted data. SAb, JJA, BG, MJH, DH, DK, AL, MLi, JL, PN, AOli, LO, DSc, MT, and JV wrote the manuscript. All authors reviewed manuscript drafts, approved the final version of the manuscript, decided to publish the manuscript, and agreed to be accountable for all aspects of the work.

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Declaration of interests

PAl's institute has received grants from the Catalan Government, the Spanish Government, Medicines for Malaria Venture, and the Bill & Melinda Gates Foundation. PAl has received personal fees from Medicines for Malaria Venture. DCM, CMai, PN, and LO's institutes have received grants from MVI for other malaria studies. AMo has received personal fees from Medicines for Malaria Venture and GlaxoSmithKline. CO and KO's institutes have received grants from the Malaria Clinical Trial Alliance. LO has received financial support from GlaxoSmithKline to participate to scientific congresses and to set up the "Trust in Science" grant. MT is a board member of the Optimus Foundation, and his institution is reimbursed for his activities on the Scientific Advisory Board of the Novartis Institute for Tropical Diseases. MT has also received for his institution other grants from MVI and from the Bill & Melinda Gates Foundation, and travel reimbursements from MVI and Sanaria. WRB, JCoh, YG, DH, EJ, DLa, AL, MLi, OO-A, AOli, and JV are, or were at the time of the study, employed by the GlaxoSmithKline group of companies. JCoh is an independent consultant for GSK Vaccines. JCoh, EJ, DLa, and OO-A have shares or stock options in the GlaxoSmithKline group of companies. JCoh and WRB are named inventors on patents for which the rights have been assigned to GlaxoSmithKline group of companies. DK, DLe, CO, and BS are or were at the time of the study employees at PATH MVI. DSc is employed by the London School of Hygiene & Tropical Medicine, and his consultancy activities for the MVI are funded as a grant to the London School of Hygiene & Tropical Medicine by MVI. All other members of the RTS,S Clinical Trials Partnership declare no competing interests.

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