ORIGINAL ARTICLE

A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants

The RTS,S Clinical Trials Partnership

ABSTRACT

BACKGROUND

The candidate malaria vaccine RTS,S/AS01 reduced episodes of both clinical and severe malaria in children 5 to 17 months of age by approximately 50% in an ongoing phase 3 trial. We studied infants 6 to 12 weeks of age recruited for the same trial.

METHODS

We administered RTS,S/AS01 or a comparator vaccine to 6537 infants who were 6 to 12 weeks of age at the time of the first vaccination in conjunction with Expanded Program on Immunization (EPI) vaccines in a three-dose monthly schedule. Vaccine efficacy against the first or only episode of clinical malaria during the 12 months after vaccination, a coprimary end point, was analyzed with the use of Cox regression. Vaccine efficacy against all malaria episodes, vaccine efficacy against severe malaria, safety, and immunogenicity were also assessed.

RESULTS

The incidence of the first or only episode of clinical malaria in the intention-to-treat population during the 14 months after the first dose of vaccine was 0.31 per person-year in the RTS,S/AS01 group and 0.40 per person-year in the control group, for a vaccine efficacy of 30.1% (95% confidence interval [CI], 23.6 to 36.1). Vaccine efficacy in the per-protocol population was 31.3% (97.5% CI, 23.6 to 38.3). Vaccine efficacy against severe malaria was 26.0% (95% CI, –7.4 to 48.6) in the intention-to-treat population and 36.6% (95% CI, 4.6 to 57.7) in the per-protocol population. Serious adverse events occurred with a similar frequency in the two study groups. One month after administration of the third dose of RTS,S/AS01, 99.7% of children were positive for anti-circumsporozoite antibodies, with a geometric mean titer of 209 EU per milliliter (95% CI, 197 to 222).

CONCLUSIONS

The RTS,S/AS01 vaccine coadministered with EPI vaccines provided modest protection against both clinical and severe malaria in young infants. (Funded by GlaxoSmithKline Biologicals and the PATH Malaria Vaccine Initiative; RTS,S ClinicalTrials.gov number, NCT00866619.)

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Considerable gains have been achieved in malaria control during the past decade.[1,2] Nonetheless, malaria remains a major public health concern. In 2010, an estimated 216 million cases of malaria and 655,000 malaria-related deaths occurred, with the vast majority of deaths occurring in African children.[1]

The RTS,S/AS01 candidate malaria vaccine targets the pre-erythrocytic stage of the *Plasmodium falciparum* parasite. It was developed to reduce clinical and severe malaria in African children. Ideally, it would be administered through the well-established Expanded Program on Immunization (EPI).

In 2011, we reported the results for the first coprimary end point from an ongoing phase 3 trial, which showed that during 12 months of follow-up, RTS,S/AS01 had an efficacy against clinical and severe malaria of 55.8% (97.5% confidence interval [CI], 50.6 to 60.4) and 47.3% (95% CI, 22.4 to 64.2), respectively, among children 5 to 17 months of age at enrollment (per-protocol analysis).[3] Vaccine efficacy against severe malaria among children 6 to 12 weeks of age and those 5 to 17 months of age combined was 34.8% (95% CI, 16.2 to 49.2) during an average of 11 months of follow-up (range, 0 to 22). We now report on the second coprimary end point from the same trial: efficacy against clinical malaria during 12 months of follow-up among infants 6 to 12 weeks of age at enrollment, when RTS,S/AS01 was coadministered with EPI vaccines.

Methods

Study Design

Details of the study methods have been described previously[3-7] and are provided in the Supplementary Appendix and the study protocol, both of which are available with the full text of this article at NEJM.org. This phase 3, randomized, controlled, double-blind trial is being conducted at 11 centers in 7 African countries with a range of malaria-transmission intensity (Fig. S1 in the Supplementary Appendix). The trial is designed to evaluate vaccine efficacy, safety, and immunogenicity for 32 months after the first dose of study vaccine in children 6 to 12 weeks of age or 5 to 17 months of age at enrollment. The trial includes three study groups in each age category: infants who received three doses of RTS,S/AS01 administered at 1-month intervals and a booster dose 18 months after the third dose, infants who received three doses of RTS,S/AS01 at 1-month intervals without a booster dose, and a control group of infants who received a non-malaria comparator vaccine. The analysis described in this report combines the first two groups (referred to as the RTS,S/AS01 group) and compares this group with the control group[6] 14 months after the first dose of vaccine administered in children 6 to 12 weeks of age (Fig. S2 in the Supplementary Appendix). The trial protocol was approved by all relevant ethics review boards and national regulatory authorities (Tables S1A and S1B in the Supplementary Appendix). Written informed consent was obtained from the children's parents or guardians. The study was undertaken in accordance with Good Clinical Practice guidelines.[8]

Study Oversight

The trial was sponsored by GlaxoSmithKline Biologicals (GSK), the vaccine developer and manufacturer, and funded by both GSK and the Program for Appropriate Technology in Health (PATH) Malaria Vaccine Initiative, which received a grant from the Bill and Melinda Gates Foundation. All study centers received study grants from the Malaria Vaccine Initiative, which also provided funding for authors' travel and accommodations related to this trial. All the authors reviewed all manuscript drafts, approved the final version of the manuscript, and made the decision to submit it for publication. No GSK authors were involved in the collection or analysis of the data; the analysis was performed by an independent statistician. The authors had full access to the results. The authors remain unaware of study-group assignments in this ongoing trial and do not have access to the raw data at this point. Details of the contributions of all the authors to the study are available in the Supplementary Appendix. The Clinical Trials Partnership Committee and Writing Group vouch for the completeness and accuracy of the data presented and for the fidelity of this report to the study protocol.

Randomization and Vaccination

From December 2009 through January 2011, a total of 6537 infants 6 to 12 weeks of age were randomly assigned to one of the three study groups in a 1:1:1 ratio. Three doses of the RTS,S/AS01 or

the comparator vaccine, meningococcal serogroup C conjugate vaccine (Menjugate, Novartis), were coadministered with EPI vaccines according to the World Health Organization EPI schedule.[9] EPI vaccines comprised a diphtheria–tetanus–whole-cell pertussis–hepatitis B–*Hemophilus influenzae* type b pentavalent vaccine (Tritanrix HepB Hib, GSK) and an oral poliovirus vaccine containing serotypes 1, 2, and 3 (Polio Sabin, GSK). The study and pentavalent vaccines were administered intramuscularly at different protocol-specified injection sites.

SURVEILLANCE FOR CLINICAL AND SEVERE MALARIA

Passive surveillance for malaria began at the time of the first vaccination. Parents or guardians of the study participants were encouraged to seek care at a health facility if the child had any signs of illness, and transportation was facilitated. All participants who presented to a study facility with reported or documented fever during the previous 24 hours were evaluated for malaria.

The primary efficacy end point for this analysis was the incidence of clinical malaria, defined as an illness in a child who was brought to a study facility with an axillary temperature of 37.5°C or higher and *P. falciparum* asexual parasitemia at a density of more than 5000 parasites per cubic millimeter or a case of malaria meeting the primary case definition of severe malaria (Table S2 in the Supplementary Appendix). Different parasite thresholds were used for secondary case definitions (Table 1). Participants who were hospitalized were evaluated for severe malaria on the basis of a protocol-defined algorithm (Table S3 in the Supplementary Appendix).[4,10]

SAFETY SURVEILLANCE

Data regarding serious adverse events were recorded by means of passive surveillance beginning after the first dose of vaccine. Verbal autopsies were conducted for deaths that occurred outside study facilities.[11] Information was collected on all unsolicited reports of adverse events that occurred within 30 days after vaccination and on reactogenicity (pain, swelling, redness at the injection site, drowsiness, fever, irritability or fussiness, or loss of appetite) within 7 days after vaccination among the first 200 participants enrolled at each center. Symptom intensity was assessed with the use of standardized methods (Table S4 in the Supplementary Appendix). Information on related adverse events within 30 days after vaccination was collected for all participants. Study clinicians used clinical judgment to decide whether an adverse event was likely to be related to the vaccine. In an analysis of previous RTS,S studies, rash was observed more frequently in children vaccinated with RTS,S than in controls.[12] Rashes and mucocutaneous diseases occurring within 30 days after vaccination and seizures occurring within 7 days after vaccination were reported according to Brighton Collaboration guidelines[13,14] (see the Methods section in the Supplementary Appendix).

IMMUNOGENICITY

Anti-circumsporozoite antibodies were measured by means of enzyme-linked immunosorbent assay[15] in the first 200 infants enrolled at each study center at screening and 1 month after dose-3. An antibody titer of 0.5 EU per millimeter or greater was considered to be positive.

LABORATORY AND RADIOLOGIC PROCEDURES

Laboratory and radiologic procedures have been reported previously[5] and are described in the Supplementary Appendix.

STATISTICAL ANALYSIS

The statistical methods have been described in detail previously.[3,7] We used Cox regression models (1 minus hazard ratio) to evaluate vaccine efficacy against the first or only episode of clinical malaria, using the study center as a stratification factor that allowed for differential baseline hazards. For the coprimary end point, vaccine efficacy against clinical malaria during 12 months of follow-up in the two age categories, 97.5% confidence intervals were used, ensuring an overall two-sided alpha level of 5%. The proportionality of hazards was evaluated by means of Schoenfeld residuals and models, including time-varying covariates. Secondary analyses, which included evaluations based on other case definitions and an analysis including multiple episodes of clinical malaria, were performed with the use of negative binomial regression. Vaccine efficacy against severe malaria was defined as 1 minus the risk ratio and is presented with 95% confidence intervals and Fisher's exact P values.

Primary analyses of vaccine efficacy were based on the per-protocol population, which included all participants who received three doses

Table 1. Efficacy of the RTS,S/AS01 Vaccine against Clinical and Severe Malaria in Infants Enrolled at 6 to 12 Weeks of Age.

Variable	RTS,S/AS01 Vaccine			Control Vaccine			Protective Efficacy		Protective Efficacy Adjusted for Covariates*	
	No. of Events	Person-Yr	Event Rate	No. of Events	Person-Yr	Event Rate	% (CI)†	P Value	% (95% CI)	P Value
Clinical malaria‡										
Per-protocol population (12 mo after third dose of vaccine)										
First or only episode										
>5000 parasites/mm³ and temperature ≥37.5°C (coprimary end point)	1161	3163	0.37	714	1476	0.48	31.3 (23.6–38.3)	<0.001	31.5 (24.7–37.6)	<0.001
>0 parasites/mm³ and measured or reported fever	1475	2921	0.50	879	1328	0.66	32.4 (26.5–37.9)	<0.001	32.6 (26.7–38.0)	<0.001
>500 parasites/mm³ and temperature ≥37.5°C	1282	3073	0.42	770	1429	0.54	30.3 (23.7–36.2)	<0.001	30.4 (23.8–36.3)	<0.001
>20,000 parasites/mm³ and temperature ≥37.5°C	1005	3256	0.31	630	1535	0.41	31.4 (24.2–37.9)	<0.001	31.6 (24.4–38.1)	<0.001
All episodes, >5000 parasites/mm³ and temperature ≥37.5°C	2301	3604	0.64	1626	1790	0.91	32.9 (26.3–38.8)	<0.001	33.0 (26.4–38.9)	<0.001
Intention-to-treat population (14 mo after first dose of vaccine)										
First or only episode, >5000 parasites/mm³ and temperature ≥37.5°C	1283	4106	0.31	782	1949	0.40	30.1 (23.6–36.1)	<0.001		
All episodes, >5000 parasites/mm³ and temperature ≥37.5°C	2615	4688	0.56	1864	2345	0.79	32.9 (26.7–38.5)	<0.001		
	No. of Infants	No. Affected	Affected rate	No. of Infants	No. Affected	Affected rate	% (95% CI)	P Value		
			%			%				
Severe malaria§										
Per-protocol population (12 mo after third dose of vaccine)										
Primary case definition	3995	58	1.5	2008	46	2.3	36.6 (4.6–57.7)	0.02		
Secondary case definition	3995	63	1.6	2008	51	2.5	37.9 (8.3–57.8)	0.01		
Intention-to-treat population (14 mo after first dose of vaccine)										
Primary case definition	4358	77	1.8	2179	52	2.4	26.0 (–7.4–48.6)	0.09		
Secondary case definition	4358	83	1.9	2179	58	2.7	28.4 (–1.9–49.4)	0.06		

* In the adjusted analyses, data were stratified according to study site with adjustment for the distance to the nearest outpatient health facility.

† All end points are presented with 95% confidence intervals except for the coprimary end point, which is presented with 97.5% confidence intervals. The coprimary end point was defined as vaccine efficacy against a first or only episode of clinical malaria, according to the primary case definition.

‡ The primary case definition of clinical malaria was an illness in a child brought to a study facility with a temperature of ≥37.5°C and *Plasmodium falciparum* asexual parasitemia at a density of >5000 parasites per cubic millimeter or a case of malaria meeting the primary case definition of severe malaria.

§ The primary case definition of severe malaria was *P. falciparum* asexual parasitemia at a density of >5000 parasites per cubic millimeter with one or more markers of disease severity and without diagnosis of a coexisting illness. The secondary case definition of severe malaria was *P. falciparum* asexual parasitemia at a density of >5000 parasites per cubic millimeter with one or more markers of disease severity, including cases in which a coexisting illness was present or could not be ruled out. Markers of severe disease were prostration, respiratory distress, a Blantyre coma score of ≤2 (on a scale of 0 to 5, with higher scores indicating a higher level of consciousness), two or more observed or reported seizures, hypoglycemia, acidosis, elevated lactate level, or hemoglobin level of <5 g per deciliter. Coexisting illnesses were defined as radiographically proven pneumonia, meningitis on analysis of cerebrospinal fluid, bacteremia, or gastroenteritis with severe dehydration.

of a study vaccine coadministered with EPI vaccines and who were included in efficacy surveillance, starting 14 days after the third dose of a study vaccine. The modified intention-to-treat population included all participants who received at least one dose of a study vaccine. In the adjusted analyses, vaccine efficacy was adjusted for study center and distance to the nearest outpatient facility (≤5 km vs. >5 km). Data were censored 14 months after the first dose of vaccine, or at the date of emigration, withdrawal of consent, or death.

Serious adverse events were coded from clinician-assigned diagnoses according to the preferred terms of the *Medical Dictionary for Regulatory Activities*[16] and were based on available clinical and laboratory evidence.

The primary analysis of immunogenicity was based on the per-protocol population. Anti-circumsporozoite antibody titers were plotted and evaluated after the third dose of a study vaccine on the basis of seropositivity levels and geometric mean titers.

RESULTS

STUDY POPULATION

In total, 6537 infants 6 to 12 weeks of age were enrolled; 6003 (91.8%) were included in the per-protocol analysis (Fig. 1, and Fig. S3 in the Supplementary Appendix). Baseline demographic characteristics were similar in the two study groups (Table S5 in the Supplementary Appendix). The numbers of participants and malaria episodes according to study center are shown in Table S6 in the Supplementary Appendix. As expected, the majority of malaria episodes were reported by centers in areas with the highest transmission; 43.5% of all clinical malaria episodes were reported by two high-transmission sites in western Kenya. These two sites, combined with the site in Nanoro, Burkina Faso (where transmission is high but seasonal), accounted for 72.6% of clinical malaria episodes in this analysis. The rate of use of insecticide-treated nets was 85.8% overall and was similar in the two study groups. Indoor residual spraying was conducted as a public health intervention at four study centers; at those centers, spraying coverage was low (Table S7 in the Supplementary Appendix).

VACCINE EFFICACY AGAINST CLINICAL AND SEVERE MALARIA

In the per-protocol population, the incidence of a first or only episode of clinical malaria meeting the primary case definition during 12 months of follow-up was 0.37 per person-year in the RTS,S/AS01 group and 0.48 per person-year in the control group, for a vaccine efficacy of 31.3% (97.5% CI, 23.6 to 38.3). Kaplan–Meier curves are shown in Figures 2A and 2B. Vaccine efficacy was not constant over time ($P<0.001$ by Schoenfeld residuals), with efficacy higher at the beginning than at the end of the follow-up period (Table S8 in the Supplementary Appendix). Vaccine efficacy against all clinical malaria episodes was 32.9% (95% CI, 26.3 to 38.8). Estimates of efficacy against clinical malaria were consistent across all case definitions and in both adjusted and intention-to-treat analyses (Table 1).

At least one episode of severe malaria occurred in 58 of 3995 infants (1.5%) in the RTS,S/AS01 group and in 46 of 2008 infants (2.3%) in the control group, for a vaccine efficacy of 36.6% (95% CI, 4.6 to 57.7) in the per-protocol population. In the intention-to-treat population, at least one episode of severe malaria occurred in 77 of 4358 infants (1.8%) in the RTS,S/AS01 group and in 52 of 2179 infants (2.4%) in the control group, for a vaccine efficacy of 26.0% (95% CI, –7.4 to 48.6) (Table 1, and Tables S15 and S16 in the Supplementary Appendix).

SAFETY

Serious Adverse Events

Serious adverse events were reported in 17.9% (95% CI, 16.8 to 19.1) of recipients of the RTS,S/AS01 vaccine and in 19.2% (95% CI, 17.6 to 20.9) of recipients of the meningococcal vaccine (Table 2, and Table S9 in the Supplementary Appendix). A total of 94 infants died: 66 of 4358 infants (1.5%; 95% CI, 1.2 to 1.9) in the RTS,S/AS01 group and 28 of 2179 infants (1.3%; 95% CI, 0.9 to 1.9) in the control group. Causes of death were similar in the two groups; none of the deaths were thought to be related to vaccination (Table S10 in the Supplementary Appendix). Serious adverse events that were considered to be related to a study vaccine occurred in 7 infants: 4 of the 4358 infants in the RTS,S/AS01 group and 3 of the 2179 infants in the control group; 4 events (2 in each group)

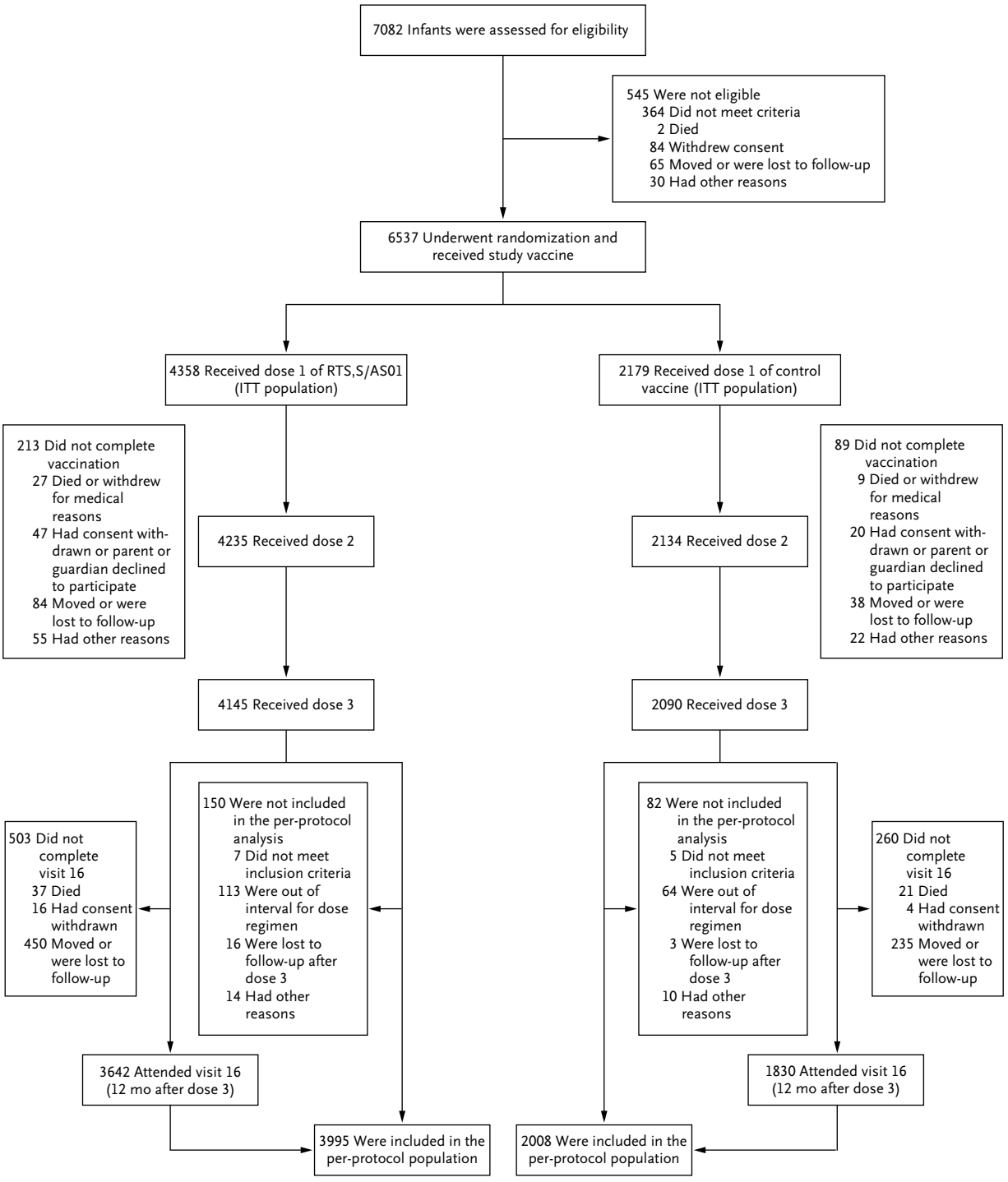

Figure 1. Enrollment of Infants 6 to 12 Weeks of Age.

ITT denotes intention to treat.

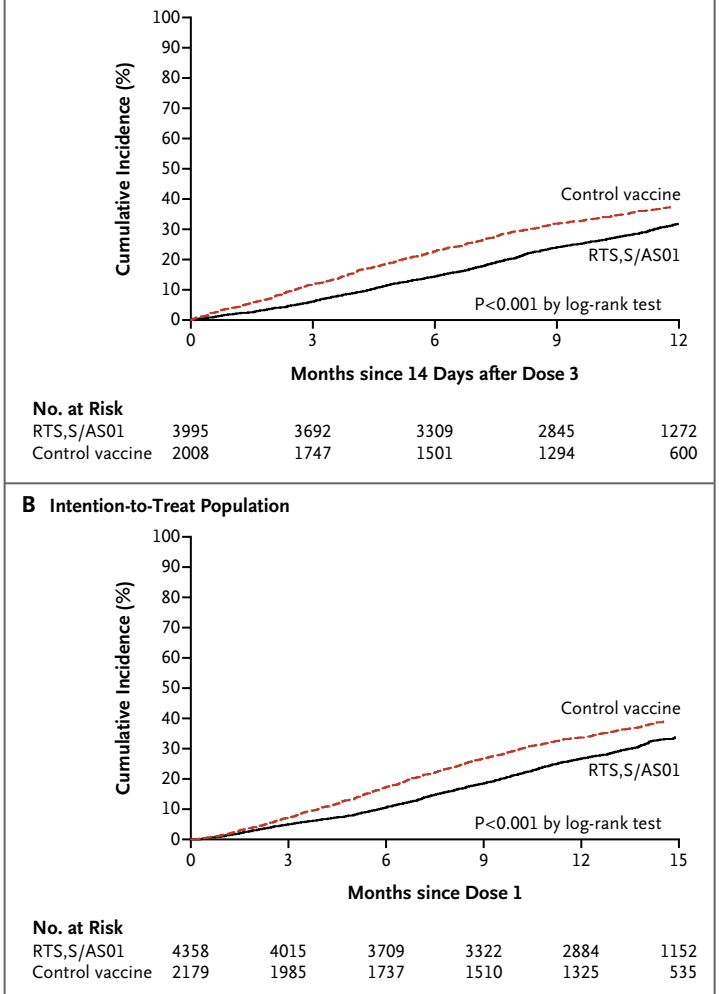

Figure 2. Cumulative Incidence of a First or Only Episode of Clinical Malaria (Primary Case Definition).

The cumulative incidence of clinical malaria in infants 6 to 12 weeks of age at enrollment is shown for the 12 months of follow-up after the third dose of a study vaccine in the per-protocol population (Panel A) and for the 14 months of follow-up after the first dose of vaccine in the intention-to-treat population (Panel B).

were episodes of fever for which infants were hospitalized for investigation. One infant (in the control group) had anaphylaxis, one infant (in the RTS,S/AS01 group) had a suspected injection-site infection related to the pentavalent vaccine, and one infant (in the RTS,S/AS01 group) had repeated febrile seizures associated with a respiratory infection. The frequency of seizures within 7 days after vaccination, reported previously, was similar in the two study groups.[3]

Meningitis of any cause was reported as a serious adverse event in 11 infants: 9 of the 4358 infants in the RTS,S/AS01 group and 2 of the 2179 infants in the control group (relative risk in the RTS,S/AS01 group, 2.3; 95% CI, 0.5 to 10.4). A pathogen was identified for 7 of the events (salmonella in 3 episodes of meningitis and pneumococcus in 4 episodes). The 4 remaining events, with no pathogen identified, were reported by a single study center (3 episodes of meningitis in the RTS,S/AS01 group and 1 episode in the control group). Of the 11 episodes of meningitis, 2 were new (1 due to pneumococcus and 1 due to salmonella); the 9 other episodes have been reported previously.[3] Investigator-driven medical review of previously reported meningitis episodes led to reclassification of 1 episode as an episode of pneumonia and reclassification of 4 episodes without cause as 2 episodes of pneumococcal meningitis and 2 of salmonella meningitis. Four of the episodes of meningitis occurred within 30 days after vaccination.

Adverse Events

Unsolicited reports of adverse events within 30 days after vaccination were recorded with similar frequency in the RTS,S/AS01 group (79.4%; 95% CI, 77.2 to 81.5) and in the control group (81.3%; 95% CI, 78.3 to 84.1). No clinically important imbalances were observed (Table S11A in the Supplementary Appendix). Information on unsolicited reports of adverse events related to the vaccine or leading to withdrawal within 30 days after vaccination is shown in Table S11B in the Supplementary Appendix. The frequency of solicited reports of local symptoms was similar among infants who received the RTS,S/AS01 vaccine and among those who received the meningococcal vaccine and was lower than that observed with the pentavalent vaccine (Table S13 in the Supplementary Appendix). Systemic reactogenicity was higher in the RTS,S/AS01 group than in the control group (Fig. 3, and Table S12 in the Supplementary Appendix). Postvaccination fever was reported after 30.6% of doses (95% CI, 29.2 to 32.0) in the RTS,S/AS01 group and after 21.1% of doses (95% CI, 19.4 to 22.8) in the control group. A temperature higher than 39°C was reported after less than 1% of doses. The incidence of mucocutaneous disease was similar in the two study groups (Table S14 in the Supplementary Appendix).

Table 2. Serious Adverse Events in Infants 6 to 12 Weeks of Age at Enrollment during 14 Months after the First Dose of Vaccine (Intention-to-Treat Population).

Variable	RTS,S/AS01 Vaccine (N=4358)		Control Vaccine (N=2179)	
	No. of Infants	% (95% CI)	No. of Infants	% (95% CI)
Serious events in all infants				
≥1 Serious adverse event	782	17.9 (16.8–19.1)	419	19.2 (17.6–20.9)
≥1 Serious adverse event, excluding malaria	760	17.4 (16.3–18.6)	407	18.7 (17.1–20.4)
≥1 Fatal serious adverse event*	66	1.5 (1.2–1.9)	28	1.3 (0.9–1.9)
≥1 Serious adverse event related to vaccine	4	0.1 (0.0–0.2)	3	0.1 (0.0–0.4)
≥1 Serious adverse event within 30 days after vaccination	192	4.4 (3.8–5.1)	96	4.4 (3.6–5.4)
Events with an incidence ≥0.5%†				
Pneumonia	302	6.9 (6.2–7.7)	152	7.0 (5.9–8.1)
Gastroenteritis	260	6.0 (5.3–6.7)	139	6.4 (5.4–7.5)
Malaria	184	4.2 (3.6–4.9)	115	5.3 (4.4–6.3)
Anemia	90	2.1 (1.7–2.5)	58	2.7 (2.0–3.4)
Febrile convulsion	82	1.9 (1.5–2.3)	46	2.1 (1.5–2.8)
Bronchiolitis	28	0.6 (0.4–0.9)	21	1.0 (0.6–1.5)
Convulsion	41	0.9 (0.7–1.3)	19	0.9 (0.5–1.4)
Bronchopneumonia	35	0.8 (0.6–1.1)	20	0.9 (0.6–1.4)
Upper respiratory tract infection	36	0.8 (0.6–1.1)	19	0.9 (0.5–1.4)
Salmonella sepsis	26	0.6 (0.4–0.9)	16	0.7 (0.4–1.2)
Malnutrition	29	0.7 (0.4–1.0)	7	0.3 (0.1–0.7)
Sepsis	26	0.6 (0.4–0.9)	10	0.5 (0.2–0.8)
HIV infection‡	27	0.6 (0.4–0.9)	9	0.4 (0.2–0.8)
Enteritis	11	0.3 (0.1–0.5)	12	0.6 (0.3–1.0)
Urinary tract infection	16	0.4 (0.2–0.6)	10	0.5 (0.2–0.8)
Measles	20	0.5 (0.3–0.7)	7	0.3 (0.1–0.7)
Pyrexia	15	0.3 (0.2–0.6)	11	0.5 (0.3–0.9)

* More than one fatal serious adverse event could be attributed to a single infant if there was more than one underlying cause of death (e.g., meningitis and sepsis).
† Events are listed according to the preferred terms in the *Medical Dictionary for Regulatory Activities*.
‡ HIV denotes human immunodeficiency virus.

IMMUNOGENICITY

Before vaccination, 34.3% and 35.2% of infants in the RTS,S/AS01 and control groups, respectively, were positive for anti-circumsporozoite antibodies but at low titers (Fig. S4 in the Supplementary Appendix). One month after the third dose of the study vaccine, 99.7% of infants in the RTS,S/AS01 group were positive for anti-circumsporozoite antibodies, with a geometric mean titer of 209 EU per milliliter (95% CI, 197 to 222).

DISCUSSION

This phase 3 trial showed that in young infants, the RTS,S/AS01 candidate vaccine provided modest protection against malaria when coadministered with EPI vaccines. The efficacy of RTS,S/AS01 reported here is lower than that observed in a phase 2 trial involving infants at three of the phase 3 trial sites, in which RTS,S/AS01 was coadministered with EPI vaccines. In that trial, geo-

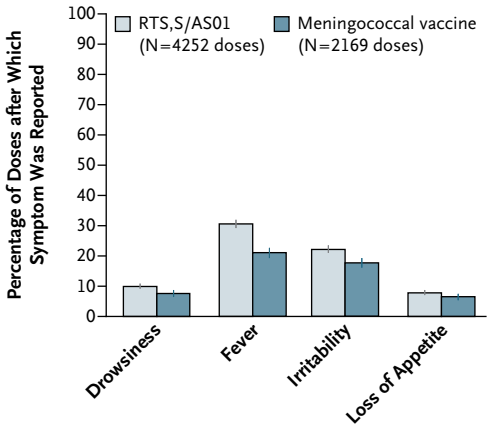

Figure 3. Incidence of Solicited Reports of Adverse Events during the 7-Day Postvaccination Period after Each Dose (Intention-to-Treat Population).

Solicited reports of adverse events during the 7-day postvaccination period were recorded and analyzed in the first 200 infants enrolled at each study site.

metric mean titers of anti-circumsporozoite antibodies after vaccination were similar to those measured here, but vaccine efficacy against clinical malaria was 61.6% (95% CI, 35.6 to 77.1).[17] Although we wish to avoid overinterpretation of the results of this previously reported small phase 2 trial with wide confidence intervals, it is notable that this higher estimate of efficacy comes from a study conducted at sites in areas with low-to-moderate malaria transmission. It is possible that the pooled estimate across the 11 centers in the phase 3 trial obscures differences in vaccine efficacy according to transmission intensity and that these two sets of results are compatible with each other.

The efficacy of the RTS,S/AS01 vaccine reported here is also lower than that reported previously among older children recruited for this trial at the same study centers.[3] A likely explanation for the lower vaccine efficacy among infants is an age-dependent differential immune response to the vaccine. This concept is supported by the lower anti-circumsporozoite antibody titers observed in infants (geometric mean titer, 209 EU per milliliter; 95% CI, 197 to 222) as compared with titers in older children (621 EU per milliliter; 95% CI, 592 to 652), reported previously.[3] Although the titer of anti-circumsporozoite antibodies is not an established correlate of the level of protection, an association with efficacy has been observed in several trials.[17-21] Infants may have mounted a lower immune response than older children owing to coadministration of RTS,S/AS01 with routine EPI vaccines, an inhibitory effect of maternally derived anti-circumsporozoite antibodies, an absence of priming with hepatitis B vaccine or with *P. falciparum* infection, or the infant's immature immune system.

Coadministration of RTS,S/AS01 with the pentavalent vaccine and the oral poliovirus vaccine might have resulted in immune interference and contributed to the lower anti-circumsporozoite antibody titers in the younger infants. Two phase 2 studies have explored the immunologic response to the related RTS,S/AS02 vaccine, either when coadministered with a diphtheria–tetanus–pertussis–hepatitis B vaccine or when given 2 weeks afterward. The geometric mean titer of anti-circumsporozoite antibodies was lower when vaccines were coadministered than when they were staggered (70 EU per milliliter [95% CI, 54 to 90] vs. 200 EU per milliliter [95% CI, 151 to 265]).[20,21] However, vaccine efficacy against infection was similar in the two trials (65.2% [95% CI, 20.7 to 84.7] during 6 months after vaccination and 65.9% [95% CI, 42.6 to 79.8] during 3 months after vaccination, respectively).

An absence of priming with hepatitis B vaccine or with *P. falciparum* infection may also have contributed to the lower anti-circumsporozoite antibody titers. In this trial, infants simultaneously received a hepatitis B surface antigen (HBsAg)–containing combination vaccine and the RTS,S vaccine, which contains HBsAg fused as a carrier protein to the circumsporozoite protein. Immune interference on concurrent administration of similar protein components has been described.[22] In contrast, in older children vaccinated against hepatitis B, memory T-cell reactivation may have enhanced the anti-circumsporozoite antibody response to RTS,S/AS01.[22] One study showed a tendency toward higher anti-circumsporozoite antibody responses in children who had been vaccinated against hepatitis B than in children who had not previously received hepatitis B vaccine.[23] Maternally derived antibodies can interfere with the immune response in young infants; such interference is common with live vaccines, such as the measles vaccine, but can also occur with some protein vaccines.[24,25] Similarly, pas-

sively acquired antibodies to either HBsAg or the circumsporozoite components of the RTS,S/AS01 vaccine might have suppressed immune responses. Finally, although most protein vaccines and polysaccharide–protein conjugate vaccines are immunogenic in young infants, improved immunogenicity and efficacy have often been achieved when vaccination has extended beyond the first few months of life.[22,26,27]

As previously reported in older children,[3] statistical models indicated nonproportionality of hazards over time. This could be due to waning vaccine efficacy, differential acquisition of natural immunity, or other factors that may influence the model,[28] such as heterogeneity of exposure, the vaccine effect at the individual level, or both.[29,30] If vaccine efficacy does wane, this might contribute to the lower observed efficacy among infants than among older children, especially because young infants may be less susceptible to malaria in the immediate postvaccination period owing to maternally acquired immunity, fetal hemoglobin, lower exposure, and other factors.[31]

The 11 sites of the phase 3 trial cover a wide range of malaria-transmission intensity. The inclusion of sites in high-transmission or seasonal-transmission areas and the large proportion of cases of severe and clinical malaria from these sites might have contributed to the lower vaccine efficacy among infants in this trial than in earlier trials involving infants. The implications of the large representation of malaria episodes from high-transmission areas may become apparent when site-specific data are analyzed at a later date, as specified by the protocol. Estimates of site-specific vaccine efficacy and the corresponding estimates of clinical or severe malaria episodes averted will help to determine what role this vaccine might have in malaria control. Exploration of factors that might affect vaccine efficacy, including the effect of maternal antibodies, the role of immune interference by EPI vaccines, the effect of the RTS,S/AS01 booster, and status with respect to previous exposure to *P. falciparum* parasites, will provide crucial information for the further development of this vaccine and for other malaria vaccines under development.[32]

Overall, fatal, or vaccine-related serious adverse events were balanced between the study groups. In the previous analysis, which included infants and older children, the incidence of meningitis was imbalanced between the RTS,S/AS01 and control groups.[3] The imbalance remains, but we now have clarified that the majority of cases had a bacterial cause. We will continue to monitor the incidence of meningitis throughout the trial. The imbalance in the incidence of rash, observed in previous RTS,S studies,[12,33] was not confirmed in this larger trial.

This phase 3 trial shows efficacy of the RTS,S/AS01 vaccine. Data from the remainder of this trial and additional studies in progress will contribute to the understanding of the complex interplay among the intensity of exposure to malaria, the immune response, and vaccine efficacy.

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APPENDIX

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