

ORIGINAL ARTICLE

Four-Year Efficacy of RTS,S/AS01E and Its Interaction with Malaria Exposure

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ABSTRACT

BACKGROUND

The candidate malaria vaccine RTS,S/AS01E has entered phase 3 trials, but data on long-term outcomes are limited.

METHODS

For 4 years, we followed children who had been randomly assigned, at 5 to 17 months of age, to receive three doses of RTS,S/AS01E vaccine (223 children) or rabies vaccine (224 controls). The end point was clinical malaria (temperature of $\geq 37.5^{\circ}\text{C}$ and *Plasmodium falciparum* parasitemia density of >2500 parasites per cubic millimeter). Each child's exposure to malaria was estimated with the use of the distance-weighted local prevalence of malaria.

RESULTS

Over a period of 4 years, 118 of 223 children who received the RTS,S/AS01E vaccine and 138 of 224 of the controls had at least 1 episode of clinical malaria. Vaccine efficacies in the intention-to-treat and per-protocol analyses were 29.9% (95% confidence interval [CI], 10.3 to 45.3; $P=0.005$) and 32.1% (95% CI, 11.6 to 47.8; $P=0.004$), respectively, calculated by Cox regression. Multiple episodes were common, with 551 and 618 malarial episodes in the RTS,S/AS01E and control groups, respectively; vaccine efficacies in the intention-to-treat and per-protocol analyses were 16.8% (95% CI, -8.6 to 36.3; $P=0.18$) and 24.3% (95% CI, 1.9 to 41.6; $P=0.04$), respectively, calculated by the Andersen–Gill extension of the Cox model. For every 100 vaccinated children, 65 cases of clinical malaria were averted. Vaccine efficacy declined over time ($P=0.004$) and with increasing exposure to malaria ($P=0.001$) in the per-protocol analysis. Vaccine efficacy was 43.6% (95% CI, 15.5 to 62.3) in the first year but was -0.4% (95% CI, -32.1 to 45.3) in the fourth year. Among children with a malaria-exposure index that was average or lower than average, the vaccine efficacy was 45.1% (95% CI, 11.3 to 66.0), but among children with a malaria-exposure index that was higher than average it was 15.9% (95% CI, -11.0 to 36.4).

CONCLUSIONS

The efficacy of RTS,S/AS01E vaccine over the 4-year period was 16.8%. Efficacy declined over time and with increasing malaria exposure. (Funded by the PATH Malaria Vaccine Initiative and Wellcome Trust; ClinicalTrials.gov number, NCT00872963.)

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MALARIA REMAINS AN IMPORTANT cause of illness and death among children in sub-Saharan Africa.¹ RTS,S is the most advanced candidate malaria vaccine and has entered phase 3 trials.²

The variation in vaccine efficacy over time will be critical to public health policy decisions concerning the introduction of the vaccine. We previously conducted a phase 2 proof-of-concept trial of RTS,S/AS01E in Kilifi, Kenya, and Korogwe, Tanzania, to evaluate its safety and efficacy against episodes of *Plasmodium falciparum* malaria in children 5 to 17 months old.³ At the end of the double-blind phase (mean duration of follow-up, 7.9 months), efficacy against the first malarial episode was 53% (95% confidence interval [CI], 28 to 69; $P<0.001$),³ and at 15 months, it was 46% (95% CI, 24 to 61; $P<0.001$).⁴ Here we present data on efficacy after 4 years of follow-up in Kilifi, Kenya.

Reductions in estimated vaccine efficacy over time may reflect a waning of the vaccine-induced protective immune responses to sporozoites, delayed acquisition of natural immunity to blood-stage parasites in the RTS,S/AS01E group because of reduced exposure to blood-stage parasites in the presence of vaccine-induced immunity to sporozoites, or an artifact in survival analysis caused by microheterogeneity in malaria exposure within the cohort.⁵ To adjust for variations in malaria exposure within our cohort, we used the distance-weighted local prevalence of malaria as a marker of a person's exposure to malaria, which we refer to as the "malaria exposure index."⁶ This exposure index is a relative measure of the intensity of malaria exposure and is distinct from absolute measures, such as the prevalence of asymptomatic parasitemia or the entomologic inoculation rate. These absolute measures are frequently used to assess exposure at the population level, but using them to assess individual exposure would be very labor-intensive.

METHODS

STUDY DESIGN

The details of the study design have been described previously^{3,4} and are provided in the Supplementary Appendix and the study protocol (including the statistical analysis plan), which are available with the full text of this article at NEJM.org. The original randomized, controlled trial was conducted in Kilifi, Kenya, and in Korogwe,

Tanzania. Here we present the Kilifi data from randomization to 48 months after the third dose was administered. The original study was sponsored by GlaxoSmithKline Biologicals, which monitored the trial and managed the database for the first 12 months of follow-up.

Extended follow-up after 12 months was led by the investigators and sponsored by the Kenya Medical Research Institute–Wellcome Trust Research Programme. GlaxoSmithKline Biologicals was responsible for reporting safety events to the regulatory authorities. For details of the roles of the sponsors and investigators, see the Supplementary Appendix.

PARTICIPANTS

Children who underwent randomization in the initial study in Kilifi³ were eligible for enrollment in this extended follow-up study. The original study and its extensions were approved by the Kenya Medical Research Institute National Ethics Review Committee, the Western Institutional Review Board, and the Oxford Tropical Research Ethics Committee. Written informed consent for the extension study was obtained from the parents or guardians of all the children with the use of approved consent forms provided in Swahili or Giriama. Nonliterate parents indicated consent by using a thumbprint, and a signature was obtained from a literate witness.

STUDY PROCEDURES

Details of the study procedures are described in the Supplementary Appendix. In brief, we used active and passive surveillance methods to identify cases of clinical malaria. We collected blood samples at specified time points to look for antibodies to *P. falciparum* circumsporozoite repeat region (anti-circumsporozoite antibodies) and asymptomatic parasitemia.

MALARIA EXPOSURE

The malaria exposure index was calculated as the distance-weighted proportion of asymptomatic or symptomatic cases of malaria within a 1-km radius of each child over a 6-month interval, with the use of data from 870 children under active surveillance in the same study area as the vaccinated cohort.⁶ Children were categorized as having lower or higher exposure according to whether they were at or below the cohort mean or above the cohort mean, respectively (see the Supplementary Appendix for details).

STATISTICAL ANALYSIS

The end point was clinical malaria (temperature of $\geq 37.5^{\circ}\text{C}$ and *P. falciparum* parasitemia of more than 2500 parasites per cubic millimeter). The intention-to-treat cohort included all children who had undergone randomization and received at least one dose of vaccine. The per-protocol cohort included children who had received three doses of vaccine according to the study protocol and for whom surveillance data were available from 2 weeks after the third dose was administered. Data from each participant were censored at 4 years of follow-up. The sample was limited by the original number of children who had undergone randomization in Kilifi. On the basis of the observed cumulative incidence of clinical malaria of 60% over a period of 4 years, the study had 85% power to detect a vaccine efficacy of 30% at the 5% significance level.

Cox proportional-hazard models were used for the analysis of first malarial episodes. Multiple episodes were analyzed by means of negative binomial regression, with clustering to adjust for repeated measures, and by means of the Andersen–Gill extension of the Cox regression model for multiple-event analysis.⁷ Vaccine efficacy was defined as 1 minus the hazard ratio or the incidence-rate ratio.

Waning of vaccine efficacy was assessed by means of time-dependent interactions between the logarithm of failure time (or year of follow-up) and RTS,S/AS01E vaccination. Plots of adjusted vaccine efficacy over time were produced from the regression coefficients in the Cox and Andersen–Gill regression models.

The reduction in the incidence of malaria that was attributable to the vaccine was calculated as the difference between the incidence of malaria in the control group and the incidence in the RTS,S/AS01E group in the intention-to-treat cohort and was expressed as the number of cases averted per 100 children per year of follow-up. The cumulative number of averted cases was calculated by summing the number of averted cases for each year. Between-group differences in the prevalence of asymptomatic *P. falciparum* parasitemia were assessed with the use of Fisher's exact test.

We calculated imputed weekly anti-circumsporozoite antibody titers by using a fractional polynomial regression model of time and cross-sectional anti-circumsporozoite antibody titers. The Cox regression model with spline functions was used to assess the relation between imputed

anti-circumsporozoite antibodies as a time-varying covariate and protection against malaria. Data were analyzed with the use of Stata software, version 12.0 (StataCorp). For details of the statistical analysis, see the Supplementary Appendix.

RESULTS

STUDY PARTICIPANTS

Of the 447 children eligible for randomization in Kilifi, 223 were randomly assigned to receive the RTS,S/AS01E vaccine and 224 to receive the rabies vaccine. A total of 320 children (72%) completed 4 years of follow-up (Fig. S1 in the Supplementary Appendix).

A total of 415 children (209 children in the RTS,S/AS01E group and 206 in the control group) received all three planned doses of vaccine according to the study protocol and were included in the per-protocol analysis. Baseline characteristics were similar in the two groups. The median duration of follow-up was 47.5 months (47.7 months in the RTS,S/AS01E group and 47.1 months in the control group), with no significant difference between the two groups (Table 1).

EFFICACY AGAINST THE FIRST OR ONLY MALARIA EPISODE

In the intention-to-treat cohort, 118 first or only episodes of clinical malaria meeting the primary case definition were documented in the RTS,S/AS01E group, as compared with 138 episodes in the control group, for an unadjusted efficacy of 29.9% (95% CI, 10.3 to 45.3; $P=0.005$) by Cox regression.

In the per-protocol cohort, 111 and 130 first or only episodes of clinical malaria were documented in the RTS,S/AS01E group and the control group, respectively, for an adjusted vaccine efficacy of 32.1% (95% CI, 11.6 to 47.8; $P=0.004$) by Cox regression (Table 2). The time-dependent Cox regression model showed weak evidence of non-proportionality of the hazard associated with the RTS,S/AS01E vaccine as compared with the rabies vaccine (hazard ratio, 1.33; 95% CI, 0.98 to 1.81; $P=0.07$). A plot of adjusted vaccine efficacy over time showed a nonsignificant waning of efficacy (Fig. 1).

EFFICACY AGAINST ALL EPISODES

We used two different analyses to examine efficacy against all malarial episodes, both of which allowed for possible variations in efficacy and the

Table 1. Demographic and Clinical Characteristics at Baseline and Follow-up in the Per-Protocol Cohort.*

Characteristic	RTSS/AS01E Vaccine (N=209)	Rabies Vaccine (N=206)	Total (N=415)
Follow-up time — mo			
Median	47.7	47.1	47.5
5th–95th percentile	12.5–48.6	11.9–48.5	12.1–48.6
Age at vaccination — mo			
Median	10.0±3.6	11.0±3.4	11.0±3.5
Range	5.0–17.0	5.0–17.0	5.0–17.0
Sex — no. (%)			
Female	106 (50.7)	102 (49.5)	208 (50.1)
Male	103 (49.3)	104 (50.5)	207 (49.9)
Geographic area — no. (%)†			
1	59 (28.2)	44 (21.4)	103 (24.8)
2	48 (23.0)	54 (26.2)	102 (24.6)
3	50 (23.9)	56 (27.2)	106 (25.5)
4	52 (24.9)	52 (25.2)	104 (25.1)
Distance to dispensary — no. (%)			
<5 km	154 (73.7)	144 (69.9)	298 (71.8)
5–10 km	55 (26.3)	62 (30.1)	117 (28.2)
Exposure index‡			
Median	0.37±0.21	0.37±0.24	0.37±0.23
Unknown — no. (%)	7 (3.3)	3 (1.5)	10 (2.4)
Bed-net use — no./total no. (%)§			
Year 1	154/204 (75.5)	148/199 (74.4)	302/403 (74.9)
Year 2	94/203 (46.3)	89/189 (47.1)	183/392 (46.7)
Year 3	47/174 (27.0)	34/145 (23.4)	81/319 (25.4)
Year 4	46/156 (29.5)	37/139 (26.6)	83/295 (28.1)

* Plus–minus values are medians ±SD. There were no significant between-group differences.

† Geographic area 1 corresponded to Bodoi, Bomani, and Junju; area 2 to Gongoni, Kolewa, Mapawa, and Mwembetsungu; area 3 to Chodari, Kadzinuni, Kapecha, and Pingilikani; and area 4 to Bokini, Dindiri, Makata, and Ng'ombeni.

‡ The exposure index is the distance-weighted local prevalence of malaria and was calculated for each participant as the mean exposure over the entire follow-up period.

§ Bed-net use was a time-varying covariate in the statistical models.

malaria exposure index over time: a modified Cox-regression model (namely, the Andersen–Gill model) and a negative binomial regression model, which fitted the data significantly better than the Poisson model (chi-square=504.21 with 1 df by the likelihood-ratio test of overdispersion, $P<0.001$) (Fig. S2 in the Supplementary Appendix).

In the intention-to-treat cohort, we recorded 551 and 618 episodes of clinical malaria among 223 children in the RTS,S/AS01E group and among 224 in the control group, respectively. On the basis of these numbers, the unadjusted efficacy against multiple episodes was 16.8% (95% CI,

–8.6 to 36.3; $P=0.18$) by the Andersen–Gill model and 18.6% (95% CI, –7.2 to 38.3; $P=0.14$) by the negative binomial regression model.

In the per-protocol cohort, there were 475 and 518 episodes of clinical malaria among 209 children in the RTS,S/AS01E group and among 206 in the control group, respectively. On the basis of these numbers, the adjusted vaccine efficacy against all episodes was 24.3% (95% CI, 1.9 to 41.6; $P=0.04$) by the Andersen–Gill model and 23.5% (95% CI, –0.7 to 41.9; $P=0.06$) by the negative binomial regression model (Table 2).

Vaccine efficacy was lower at later time points,

Table 2. Efficacy of RTS,S/AS01E Vaccine against Episodes of *Plasmodium falciparum* Clinical Malaria.*

Variable	RTS,S/AS01E Vaccine				Rabies Vaccine				Efficacy (95% CI)	P Value
	No. of Participants	No. of Events	Person-yr at Risk	Event Rate	No. of Participants	No. of Events	Person-yr at Risk	Event Rate		
Intention-to-treat cohort										
First or only episode, Cox regression										
>2500 parasites/mm ³	223	118	501.9	0.24	224	138	404.9	0.34	29.9 (10.3 to 45.3)	0.005
>0 parasites/mm ³	223	128	481.6	0.27	224	141	387.2	0.36	26.7 (6.8 to 42.3)	0.01
All episodes, Andersen–Gill Cox regression										
>2500 parasites/mm ³	223	551	836.9	0.65	224	618	791	0.78	16.8 (–8.6 to 36.3)	0.18
>0 parasites/mm ³	223	632	836.9	0.75	224	705	791	0.89	16.7 (–8.8 to 36.2)	0.18
All episodes, negative binomial regression										
>2500 parasites/mm ³	223	551	836.9	0.65	224	618	791	0.78	18.6 (–7.2 to 38.3)	0.14
>0 parasites/mm ³	223	632	836.9	0.75	224	705	791	0.89	17.9 (–6.8 to 36.9)	0.14
Per-protocol cohort										
First or only episode, Cox regression										
>2500 parasites/mm ³	209	111	466.9	0.24	206	130	360.3	0.36	32.1 (11.6 to 47.8)	0.004
>0 parasites/mm ³	209	118	450.4	0.26	206	136	342.2	0.39	34.0 (15 to 48.7)	0.001
All episodes, Andersen–Gill Cox regression										
>2500 parasites/mm ³	209	475	730.8	0.65	206	518	668.9	0.77	24.3 (1.9 to 41.6)	0.04
>0 parasites/mm ³	209	542	730.8	0.74	206	592	668.9	0.88	24.1 (1.6 to 41.5)	0.04
All episodes, negative binomial regression										
>2500 parasites/mm ³	209	475	730.8	0.65	206	518	668.9	0.77	23.5 (–0.7 to 41.9)	0.06
>0 parasites/mm ³	209	542	730.8	0.74	206	592	668.9	0.88	23.8 (0.9 to 41.3)	0.04

* Vaccine efficacy was defined as 1 minus the hazard ratio or 1 minus the incidence rate ratio. Efficacy was adjusted for age, bed-net use, geographic area, malaria exposure index, and distance to dispensary in the classic Cox model. In the negative binomial and Andersen–Gill Cox models, no adjustment was made for the malaria exposure index because of its interaction effect with vaccine efficacy. Efficacy estimates were unadjusted in the intention-to-treat analysis.

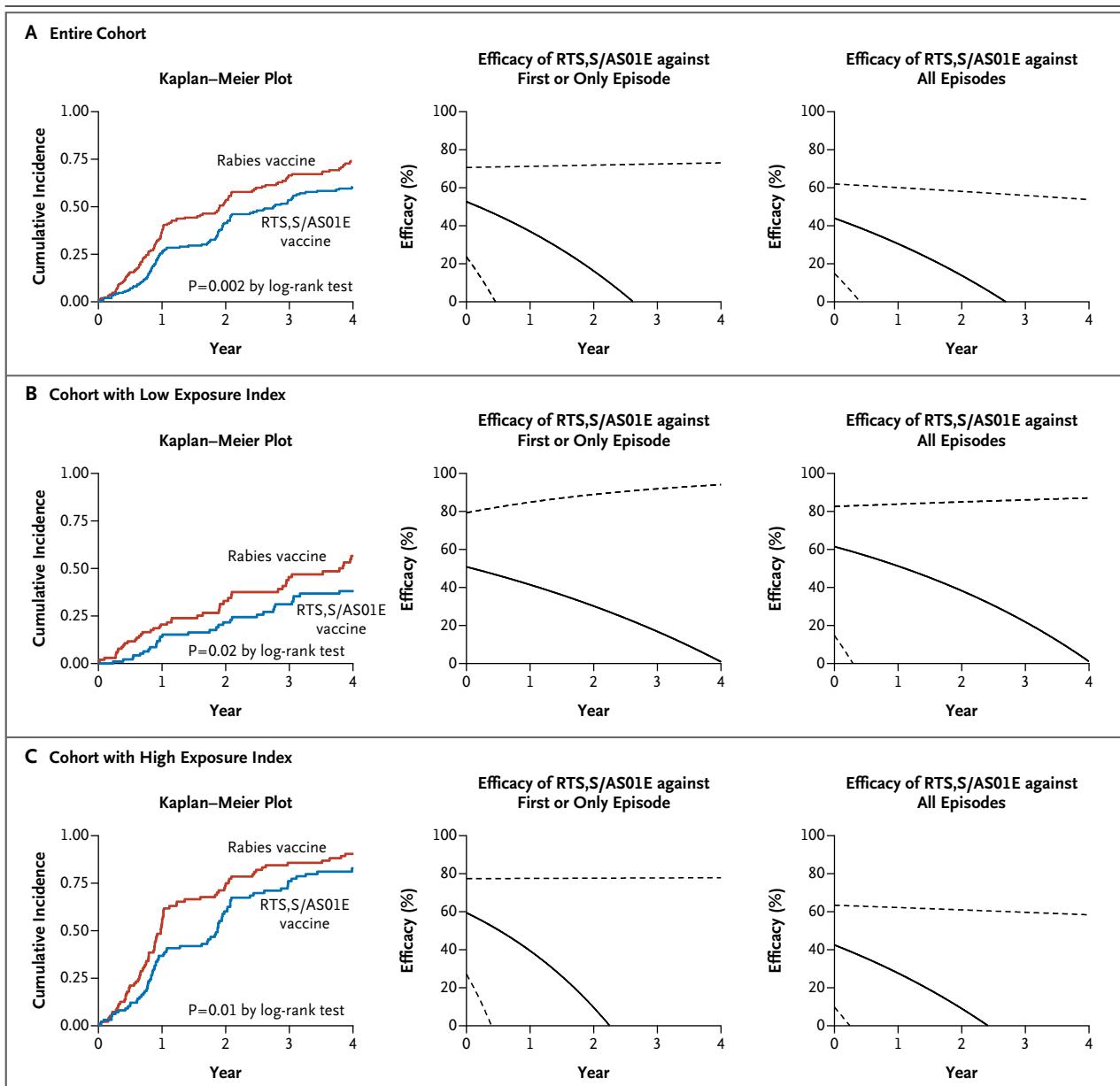


Figure 1. Kaplan–Meier Curves and Vaccine Efficacy over Time in the Per-Protocol Cohort.

Kaplan–Meier plots of the cumulative incidence of malaria and corresponding vaccine efficacy over time are shown for the entire cohort (Panel A) and for the cohorts with low and high malaria exposure indexes (Panels B and C, respectively). Clinical falciparum malaria was defined as the presence of fever (temperature $\geq 37.5^{\circ}\text{C}$) and a *Plasmodium falciparum* density of more than 2500 parasites per cubic millimeter. A log (time) interaction model was used to produce the fit for the vaccine-efficacy plots. In these plots, the solid line indicates the point estimates of efficacy and the dotted lines indicate 95% confidence intervals. Vaccine efficacy was truncated at 0% as the lower limit; hence, the lower limit of the confidence interval is visible only at the start of monitoring. The lower limit of the 95% confidence interval for vaccine efficacy against the first or only episode in the cohort with a low exposure index is not visible because it is below 0 and has been truncated.

as indicated by significant interaction between vaccine efficacy and follow-up time (hazard ratio for malaria in the RTS,S/AS01E group as compared with the control group, 1.28; 95% CI, 1.08 to 1.51; $P=0.004$ by the Andersen–Gill model; incidence-rate ratio in year 4, 1.65; 95% CI, 1.03 to 2.63; $P=0.04$ by the negative binomial regression model) (Table S1 in the Supplementary Appendix). Efficacy was lower among children with a high malaria exposure index than among those

with a low malaria exposure index, as shown by the interaction between vaccination group and the malaria-exposure index (hazard ratio in the RTS,S/AS01E group, 5.17; 95% CI, 1.98 to 13.47; $P=0.001$ by the Andersen–Gill model; incidence-rate ratio, 2.48; 95% CI, 1.18 to 5.21; $P=0.02$ by the negative binomial regression model).

Over time, the effect of malaria exposure on the risk of clinical malaria declined, as indicated by the interaction between the malaria-exposure index and follow-up time (hazard ratio in the RTS,S/AS01E group, 0.71; 95% CI, 0.51 to 0.98; $P=0.04$ by the Andersen–Gill model; incidence-rate ratio in year 3, 0.35; 95% CI, 0.15 to 0.82; $P=0.02$; and incidence-rate ratio in year 4, 0.35; 95% CI, 0.16 to 0.79; $P=0.01$ by the negative binomial regression model). There was no interaction between bed-net use and vaccination group (hazard ratio for malaria in the RTS,S/AS01E group, 0.95; 95% CI, 0.64 to 1.40; $P=0.78$).

On the basis of the negative binomial regression model, stratified efficacy estimates according to year of follow-up were 46.2% (95% CI, 21.2 to 63.4; $P=0.001$) for year 1, 24.7% (95% CI, –19.1 to 52.3; $P=0.23$) for year 2, 22.0% (95% CI, –17.0 to 48.0; $P=0.23$) for year 3, and –1.2% (95% CI, –46.8 to 31.2; $P=0.95$) for year 4 (Table S2 in the Supplementary Appendix). Vaccine efficacy was 45.1% (95% CI, 11.3 to 66.0) among children with a malaria exposure index that was average or lower than average but 15.9% (95% CI, –11.0 to 36.4) among children with a malaria exposure index that was higher than average (Table S2 in the Supplementary Appendix).

On the basis of the Andersen–Gill model, vaccine efficacy was 43.6% (95% CI, 15.5 to 62.3) in year 1 and –0.4% (95% CI, –32.1 to 45.3) in year 4 after vaccination (Fig. 1). The decline in estimated vaccine efficacy was more rapid among children with a higher malaria exposure index than among those with a lower malaria exposure index (Fig. 1), although terms for three-way interactions among vaccination group, malaria-exposure index, and time (to determine whether vaccine efficacy declined more rapidly at high exposure than at low exposure) were not significant (hazard ratio for malaria in the RTS,S/AS01E group and low malaria exposure index, 1.56; 95% CI, 0.81 to 2.99; $P=0.18$ by the Andersen–Gill model; incidence-rate ratio, 1.28; 95% CI, 0.76 to 2.14; $P=0.35$ by the negative binomial regression model).

CLINICAL MALARIA EPISODES AVERTED

The incidence of clinical malaria episodes increased in both groups during follow-up (Fig. 2A, 2B, and 2C). In the intention-to-treat cohort, the estimated numbers of malaria cases per 100 children that were averted in the 4 successive years of follow-up were 26, 22, 18, and –1, respectively, for a total of 65 cases averted over a period of 4 years (Fig. 2D). The numbers of cases averted over the 4-year follow-up period among children with a low malaria exposure index and those with a high malaria exposure index were 62 and 78 cases per 100 children, respectively (Fig. 2D).

CROSS-SECTIONAL SURVEY ANALYSIS

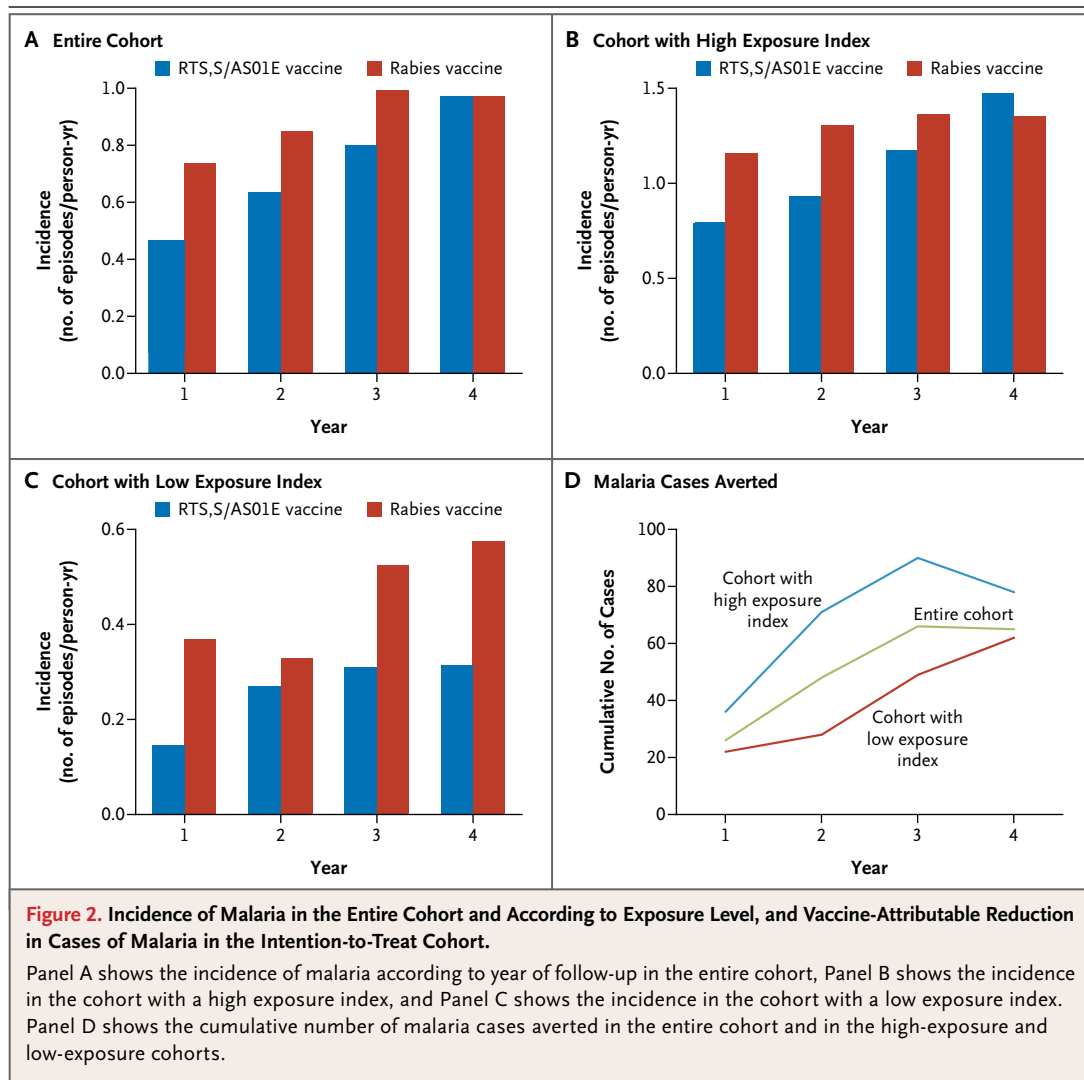
The prevalence of asymptomatic *P. falciparum* parasitemia was significantly lower among children in the RTS,S/AS01E group than among those in the control group at all cross-sectional surveys except at 8, 25, and 49 months (9% vs. 24% at 12 months, $P=0.005$; 1% vs. 6% at 15 months, $P=0.03$; and 9% vs. 20% at 38 months, $P=0.008$); the prevalence was similar in the two groups at 8 months (1% and 4%; $P=0.06$), 25 months (4% and 7%; $P=0.21$), and 49 months (7% and 5%, respectively; $P=0.47$) (Table S3 in the Supplementary Appendix). There were no significant between-group differences in the mean hemoglobin concentration.

ANTI-CIRCUMSPOROZOITE ANTIBODY TITERS AND PROTECTION

Antibody titers waned over time but remained significantly higher in the RTS,S/AS01E group than in the control group throughout the 4 years (Fig. 3A), with geometric mean titers of 17.2 enzyme-linked immunosorbent assay units (EU) per milliliter versus 2.1 EU per milliliter ($P=0.001$) at 38 months after the final vaccination. There was no significant difference in the geometric mean peak titers according to the malaria exposure index ($P=0.66$). As previously described,⁴ we found a nonlinear association between the imputed anti-circumsporozoite antibody titer and protection from clinical malaria when we included data from the full 4-year follow-up period (Fig. 3B).

DISCUSSION

During 4 years of follow-up, RTS,S/AS01E was associated with 29.9% and 16.8% efficacy against first and all episodes of *P. falciparum* clinical ma-



laria, respectively, among children vaccinated at 5 to 17 months of age in a country where malaria is endemic. Both negative binomial regression and Andersen–Gill models showed significant variations in vaccine efficacy over time and according to the level of malaria exposure (as measured by means of our malaria exposure index). The efficacy estimate during follow-up is surrounded by considerable uncertainty. On the one hand, the estimate suggests possible sustained efficacy during 4 years of follow-up. On the other hand, the significant interaction between time and vaccine efficacy argues against the hypothesis that efficacy is constant over time. Furthermore, the upper limits of the confidence intervals for efficacy in year 4 exclude an efficacy of more than 31% overall and an efficacy of more than 15% among children with a high exposure index. The waning of vaccine

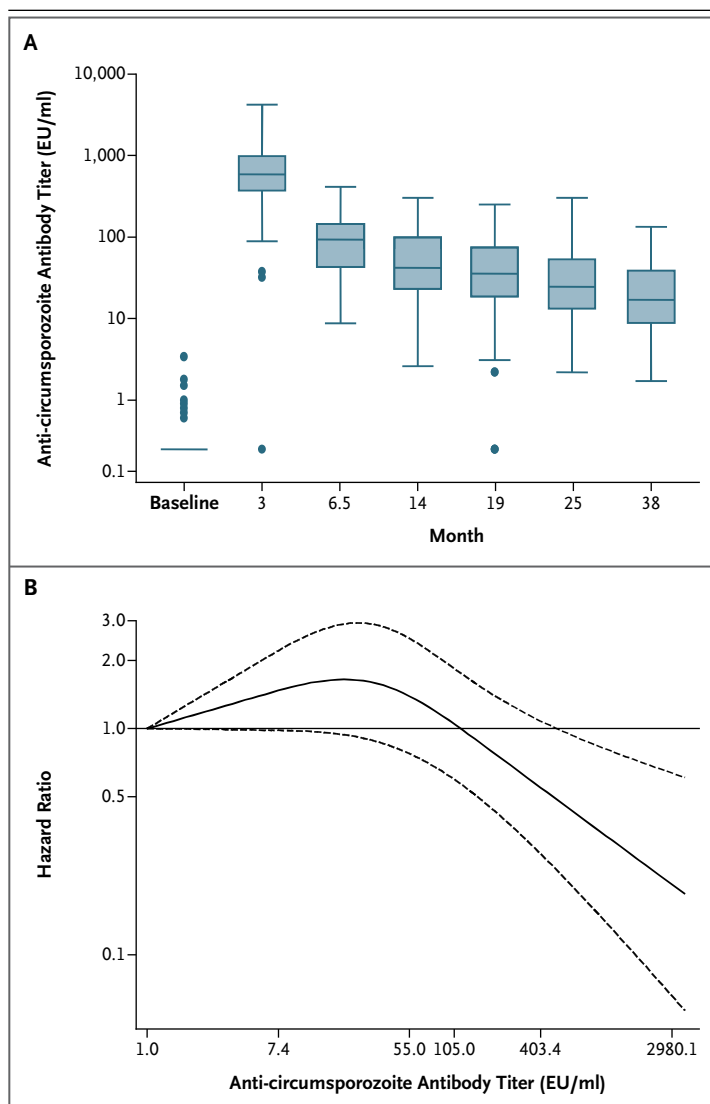
efficacy observed with our data is unlikely to be the consequence of heterogeneity in malaria exposure, because reductions in efficacy over time were similar whether first or all episodes were analyzed and also because they persisted even after the exposure index was included in the model.

We identified significant interactions between time since vaccination and vaccine efficacy and between level of exposure to malaria and vaccine efficacy. These interactions indicate how vaccine efficacy varies in the presence of other covariates. For instance, in the negative binomial regression model, the theoretical efficacy of vaccination at an exposure index of 0 and in the first year is given by an incidence-rate ratio of 0.37 (i.e., 63% efficacy). As the exposure index rises to 1, vaccine efficacy can be calculated by multiplying the incidence-rate ratio of 0.37 by the interaction

Figure 3. Anti-circumsporozoite Antibody Titers in the RTS,S/AS01E Group in the Per-Protocol Cohort.

Panel A shows anti-circumsporozoite antibody titers during follow-up in children who received the RTS,S/AS01E vaccine. Anti-circumsporozoite antibody titers in the control group were consistently low or undetectable throughout follow-up and are not shown. The horizontal line within each box represents the median, the top and bottom of each box represent the 25th and 75th percentiles, respectively, and the I bars represent the highest and lowest values within 1.5 times the interquartile range. The circles denote outliers. The horizontal line under the baseline values indicates that the values for the median and interquartile range were identical. Panel B shows the association between imputed anti-circumsporozoite antibody titers and the hazard ratio for clinical malaria episodes among children who received the RTS,S/AS01E vaccine, according to a Cox regression model with cubic splines and with a baseline titer of 1.0 enzyme-linked immunosorbent assay unit (EU) per milliliter as the reference. The dotted lines indicate the 95% confidence interval. There was no significant variation in risk between 1 EU per milliliter and 100 EU per milliliter (i.e., the confidence intervals include a hazard ratio of 1.0); at values above 100 EU per milliliter, however, there was a reduced risk of clinical malaria with increasing antibody titers.

term of the incidence-rate ratio of 2.48 (i.e., 0.91, or 9% efficacy). The graphs in Figures 2 and 3 may make these variations in efficacy intuitively evident. In contrast, a long-term follow-up study evaluating the efficacy of the related RTS,S/AS02 vaccine in Mozambican children showed no evidence of the efficacy against a first episode waning over time.⁸ One reason for the difference in these findings may be that in our study the waning efficacy was more readily apparent on analysis of all episodes in the Andersen–Gill survival models than it was with first or only episodes in the Cox regression model, and the former analysis was not used in the study in Mozambique. In addition, whereas the incidence of clinical malaria was sustained (and, in fact, increased) during follow-up of our cohort, it fell over time in Mozambique, reducing the power to identify waning efficacy. The Andersen–Gill regression model includes all malaria episodes (1169 total episodes vs. 243 first episodes in our trial) and, in particular, includes all the second, third, and fourth episodes that occurred later during follow-up but would be censored in a Cox regression analysis. Other differences between the two cohorts were higher rates of transmission in Mozambique than in Kilifi (entomologic inoculation rate of 38 vs. 22 and parasite prevalence of 20% vs. 15%), higher median age at vaccination



in Mozambique than in Kilifi (35.9 months vs. 11.0 months), and different adjuvants (AS02 in Mozambique vs. AS01 in Kilifi).

Increases in the incidence of malaria during the follow-up period reflect an increase in transmission in our study area (i.e., the Junju–Pingilikani area). The mean value of the malaria exposure index increased from 0.27 (95% CI, 0.26 to 0.28) in the first year to 0.43 (95% CI, 0.42 to 0.44) in the fourth year. This increase in the malaria exposure indexes in the Junju–Pingilikani area is in contrast with overall declines in malaria transmission in Kilifi,⁹ suggesting that there is marked regional heterogeneity in transmission within the district.^{10,11}

Estimates of vaccine efficacy were significantly lower among children with a high malaria expo-

sure index than among those with a low exposure index, suggesting that vaccine efficacy may appear to wane because the acquisition of natural immunity to blood-stage parasites among children who received the RTS,S/AS01E vaccine was slower than that among controls, owing to reduced exposure to blood-stage parasites in the RTS,S/AS01E group. Alternatively, vaccine efficacy may wane because anti-circumsporozoite antibody levels fall over time (Fig. 3A). Anti-circumsporozoite antibodies may mediate protection and were associated with a reduced risk of clinical malaria in our study (Fig. 3B). Efficacy estimates immediately after RTS,S/AS01E vaccination in the cohort with a high malaria exposure index were lower than those in the cohort with a low malaria exposure index, possibly owing to a heavy sporozoite challenge that overcomes the vaccine-induced immunity.^{12,13}

Despite waning efficacy over time and with a higher level of exposure to malaria, vaccination with RTS,S/AS01E resulted in an overall reduction in the number of episodes of clinical malaria over a 4-year follow-up period; in total, 65 cases were averted per 100 vaccinated children. Whereas vaccine efficacy determined from survival models provides a useful estimate of the biologic effect of vaccination,¹⁴ there is concern that such estimates may be misleading in public health terms.¹⁵ The absolute reductions in risk that are attributable to the vaccine provide a further metric in evaluating the possible public health effects.¹⁶

Although the confidence intervals were wide, our findings suggest that efficacy against asymptomatic parasitemia may persist longer than efficacy against clinical malaria. A similar pattern has been noted by others.⁸ A pre-erythrocytic vaccine might, in theory, achieve such an effect if blood-stage immunity was lower in persons who received the RTS,S/AS01E vaccine, as compared with those who received the rabies vaccine, thus resulting in a greater likelihood of clinical disease due to any given infection.

In conclusion, RTS,S/AS01E was associated with a reduction in the incidence of first and of all episodes of *P. falciparum* clinical malaria, but vaccine efficacy waned during the 4 years of follow-up. Both the waning of vaccine-induced immunity and the more rapid acquisition of blood-stage immunity in the controls than in the children who received the RTS,S/AS01E vaccine may have contributed to the waning of efficacy over time, and the latter may explain the variations in efficacy according to the level of malaria exposure.

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