Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial

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Summary

Background Malaria is a leading cause of morbidity and mortality worldwide. We previously reported the efficacy of the R21/Matrix-M malaria vaccine, which reached the WHO-specified goal of 75% or greater efficacy over 12 months in the target population of African children. Here, we report the safety, immunogenicity, and efficacy results at 12 months following administration of a booster vaccination.

Methods This double-blind phase 1/2b randomised controlled trial was done in children aged 5–17 months in Nanoro, Burkina Faso. Eligible children were enrolled and randomly assigned (1:1:1) to receive three vaccinations of either 5 µg R21/25 µg Matrix-M, 5 µg R21/50 µg Matrix-M, or a control vaccine (the Rabivax-S rabies vaccine) before the malaria season, with a booster dose 12 months later. Children were eligible for inclusion if written informed consent could be provided by a parent or guardian. Exclusion criteria included any existing clinically significant comorbidity or receipt of other investigational products. A random allocation list was generated by an independent statistician by use of block randomisation with variable block sizes. A research assistant from the University of Oxford, independent of the trial team, prepared sealed envelopes using this list, which was then provided to the study pharmacists to assign participants. All vaccines were prepared by the study pharmacists by use of the same type of syringe, and the contents were covered with an opaque label. Vaccine safety, efficacy, and a potential correlate of efficacy with immunogenicity, measured as anti-NANP antibody titres, were evaluated over 1 year following the first booster vaccination. The population in which the efficacy analyses were done comprised all participants who received the primary series of vaccinations and a booster vaccination. Participants were excluded from the efficacy analysis if they withdrew from the trial within the first 2 weeks of receiving the booster vaccine. This trial is registered with ClinicalTrials.gov (NCT03896724), and is continuing for a further 2 years to assess both the potential value of additional booster vaccine doses and longer-term safety.

Findings Between June 2, and July 2, 2020, 409 children returned to receive a booster vaccine. Each child received the same vaccination for the booster as they received in the primary series of vaccinations; 132 participants received 5 µg R21 adjuvanted with 25 µg Matrix-M, 137 received 5 µg R21 adjuvanted with 50 µg Matrix-M, and 140 received the control vaccine. R21/Matrix-M had a favourable safety profile and was well tolerated. Vaccine efficacy remained high in the high adjuvant dose (50 µg) group, similar to previous findings at 1 year after the primary series of vaccinations. Following the booster vaccination, 67 (51%) of 132 children who received R21/Matrix-M with low-dose adjuvant, 54 (39%) of 137 children who received R21/Matrix-M with high-dose adjuvant, and 121 (86%) of 140 children who received the rabies vaccine developed clinical malaria by 12 months. Vaccine efficacy was 71% (95% CI 60 to 78) in the low-dose adjuvant group and 80% (72 to 85) in the high-dose adjuvant group. In the high-dose adjuvant group, vaccine efficacy against multiple episodes of malaria was 78% (95% CI 71 to 83), and 2285 (95% CI 1911 to 2568) cases of malaria were averted per 1000 child-years at risk among vaccinated children in the second year of follow-up. Among these participants, at 28 days following their last R21/Matrix-M vaccination, titres of malaria-specific anti-NANP antibodies correlated positively with protection against malaria in both the first year of follow-up (Spearman's ρ –0·32 [95% CI –0·45 to –0·19]; p=0·0001) and second year of follow-up (–0·20 [–0·34 to –0·06]; p=0·02).

Interpretation A booster dose of R21/Matrix-M at 1 year following the primary three-dose regimen maintained high efficacy against first and multiple episodes of clinical malaria. Furthermore, the booster vaccine induced antibody concentrations that correlated with vaccine efficacy. The trial is ongoing to assess long-term follow-up of these participants and the value of further booster vaccinations.

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For more on the **Malaria Vaccine Implementation Programme** see https://globalhealthprogress.org/collaboration/malaria-vaccine-implementation-programme-mvip/

Introduction

Between 2019 and 2020, the number of malaria cases increased by 6% and the number of deaths increased by 12% globally, despite efforts to maintain essential malaria services during the COVID-19 pandemic. Most of these cases were in the WHO African region, with 80% of malaria deaths occurring in children younger than 5 years.[1] The 2020 milestones for morbidity and mortality outlined in the *WHO Global Technical Strategy for Malaria 2016–2030* have not been achieved, with approximately 640 000 malaria deaths reported in 2020.[1,2] It is hoped that the recent recommendation by WHO for wider use of the RTS,S/AS01 (Mosquirix; GlaxoSmithKline) malaria vaccine will encourage renewed efforts in the fight against malaria.[3]

The Malaria Vaccine Implementation Programme showed that RTS,S/AS01 has a favourable safety profile and was associated with a 30% reduction in cases of severe malaria.[3] This followed an earlier phase 3 study, where, with a median follow-up of 48 months, vaccine efficacy against clinical malaria was 36% in infants aged 5–17 months and 26% in infants aged 6–12 weeks after four doses of the vaccine.[4]

However, there is still a need to identify and develop additional malaria vaccines to allow both increased vaccine supply to ensure maximum coverage of the target population and to enable the WHO goal of a malaria vaccine candidate with 75% or greater efficacy against clinical malaria to be achieved by 2030.[5]

The R21/Matrix-M pre-erythrocytic malaria vaccine candidate was developed at the University of Oxford (Oxford, UK) and is currently manufactured by the Serum Institute of India (Pune, India).

Here, we report the ongoing safety, immunogenicity, and efficacy of R21/Matrix-M, and the number of malaria cases averted by this vaccine over 2 years of follow-up, following administration of the first booster dose.

Methods

Study design and participants

We did a phase 1/2b randomised controlled trial of the R21/Matrix-M malaria vaccine in children aged 5–17 months in Nanoro, Burkina Faso. The primary series of vaccinations were administered before or at the start of the malaria season. Results were reported previously after 12 months of follow-up, where a vaccine efficacy of 71% (95% CI 59–79) was noted in the low adjuvant dose malaria vaccine group and 77% (67–84) in the high adjuvant dose malaria vaccine group.[6] Trial methods have been described previously[6] and are summarised in appendix 2 (pp 57–97). Briefly, this

See **Online** for appendix 2

Research in context

Evidence before this study

RTS,S/AS01 (Mosquirix; GlaxoSmithKline) is the first malaria vaccine recommended by WHO for use in children in moderate-to-high transmission settings following pilot implementation trials in Ghana, Kenya, and Malawi. We searched PubMed from database inception to March 11, 2022, for published articles using the search terms "malaria vaccine" AND "clinical trial" AND "phase III" AND "efficacy". No language restrictions were applied. In a large phase 3 trial, RTS,S/AS01 had a vaccine efficacy of 68% over a period of 6 months following administration of the initial three doses, but this efficacy waned over time. At 6 months after a fourth dose, administered 18 months following the third dose, vaccine efficacy was 44% (95% CI 40–48). The most recent update to the Malaria Vaccine Technology Roadmap highlights that, by 2030, one of the goals should be to license malaria vaccines targeting *Plasmodium falciparum* that have a protective efficacy of at least 75% against clinical malaria for more than 2 years, in at-risk groups in malaria-endemic areas.

Added value of this study

This phase 1/2b randomised controlled trial reports the safety, immunogenicity, and efficacy of the R21/Matrix-M malaria vaccine at 12 months following administration of a booster dose in children aged 5–17 months in Nanoro, Burkina Faso. These findings further support our previously published efficacy data for R21/Matrix-M in the same cohort of children. We previously reported high-level efficacy of R21 adjuvanted with 50 µg of Matrix-M, administered before the malaria season, reaching the WHO-specified goal of at least 75% efficacy over 1 year in the target population of African children. The administration of a booster dose 12 months following the primary series of R21/Matrix-M vaccinations shows the added benefit of a fourth dose when administered before the malaria season. Vaccine efficacy was maintained in the high-dose adjuvant group, at 80% following the booster vaccine over 12 months, and 75% over 24 months after the primary three-dose regimen. Furthermore, vaccine efficacy against multiple episodes of clinical malaria was similar (78%) over 2 years of follow-up. R21/Matrix-M has a favourable safety profile and also induces high levels of malaria-specific anti-NANP antibodies that correlate with the observed protection against clinical malaria.

Implications of all the available evidence

These findings demonstrate the potential of a booster dose of R21/Matrix-M to maintain the high efficacy seen after the primary vaccination series. Pre-season dosing with this promising malaria vaccine candidate could provide durable protection to children living in highly seasonal malaria transmission settings. This phase 1/2b trial has now progressed to a fully enrolled phase 3 trial, with 4800 participants, aiming for licensure of the R21/Matrix-M vaccine in 2023.

study was part of the phase 2 randomised, controlled, double-blind trial conducted by the Institut de Recherche en Sciences de la Santé (IRSS) at the Clinical Research Unit of Nanoro (CRUN), Burkina Faso. Participants aged 5–17 months were recruited from the Nanoro health district and received a primary series of vaccinations consisting of three vaccine doses, 4 weeks apart, before the seasonal peak of malaria transmission (May 7–June 13, 2019). Booster doses were administered intramuscularly approximately 12 months later (June 2–July 2, 2020) to eligible participants. Exclusion criteria included any existing clinically significant comorbidity or receipt of other investigational products; the full list of inclusion and exclusion criteria is summarised in appendix 2 (p 67).

The trial was approved by the Comité d'Ethique pour la Recherche en Santé, Burkina Faso (CERS; reference number 2019-01-012), and by the national regulatory authority, Agence National de Régulation Pharmaceutique, Burkina Faso (ANRP; reference number 5005420193EC0000). Ethical approval was also granted in the UK by the Oxford Tropical Research Ethics Committee (OxTREC; reference number 19-19).

Randomisation and masking

Children aged 5–17 months were randomly assigned (1:1:1) to three groups at the start of the trial from a random allocation list, by use of block randomisation with variable block sizes. Thereafter, a research assistant from the University of Oxford, independent of the trial team, prepared sealed envelopes using this list, which was then provided to the study pharmacists to assign participants, once all the eligibility criteria had been met. All vaccines were prepared by the study pharmacists using the same type of syringe, and the contents of the syringe were covered with an opaque label. Each child received the same vaccination for the booster as they received in the primary series of vaccinations. Group 1

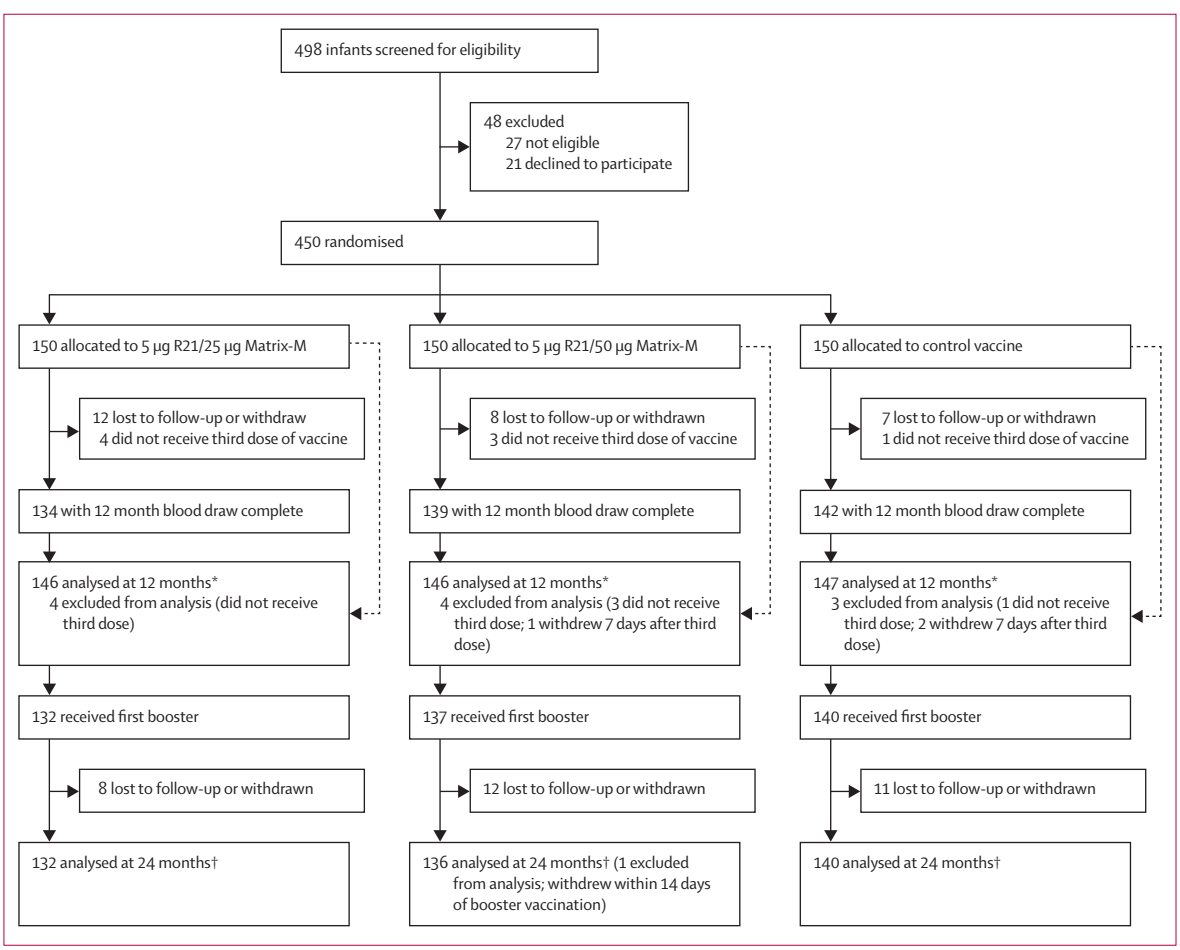

Figure 1: **Trial profile**
All participants were aged 5–17 months at enrolment. Enrolment refers to the day of the first vaccination. All participants were recruited in Nanoro, Burkina Faso, and received the same vaccination as the primary series of vaccinations for their first booster vaccination. *All participants who received the third vaccination dose were analysed for the primary outcome, since those with no event were censored at the date of the 12-month blood draw or date of withdrawal, except for three participants who withdrew within 14 days of the third vaccination. †Results were similar for the booster vaccination analysis, where one participant withdrew within 14 days of the booster vaccination.

received 5 µg R21 adjuvanted with 25 µg Matrix-M, group 2 received 5 µg R21 adjuvanted with 50 µg Matrix-M, and group 3 was the control group and received the Rabivax-S rabies vaccine. The trial was double-blinded: participants, their caregivers, and the local study team were all masked to group allocation. Only the study pharmacists preparing the vaccine had access to group allocation.

Procedures

Following the booster vaccination, local and systemic solicited adverse events, unsolicited adverse events, and serious adverse events were recorded as for the primary series of vaccinations.[6] Serious adverse events were reported descriptively.

Following the first booster vaccination, malaria was detected by passive surveillance. Treatment was initiated according to local guidelines. For the purpose of this study, the primary case definition of clinical malaria was a temperature of 37·5°C or greater, or a history of fever within the past 24 h, and *Plasmodium falciparum* parasitaemia of more than 5000 asexual forms per µL. The secondary case definition was a temperature of 37·5°C or greater, or a history of fever within the past 24 h, and *P falciparum* parasitaemia of more than 0 asexual forms per µL. Asymptomatic malaria was defined as a temperature of less than 37·5°C and *P falciparum* parasitaemia of more than 0 asexual forms per µL, and this was analysed by cross-sectional blood films at specified timepoints.

Anti-NANP antibodies were measured by ELISA, as previously described,[6] before the first booster vaccination, and at 28 days, 6 months, and 1 year following the booster vaccination dose.

Outcomes

The analysis of the original primary endpoints of the incidence of clinical malaria at 6 months following three vaccination doses and other secondary endpoints has been previously published.[6] Here, we analysed further secondary outcomes of vaccine safety, immunogenicity (measured by ELISA), and efficacy over the 12 months following the first booster vaccination and over the 24 months following the primary series of vaccinations. Participants were excluded from the efficacy analysis if they withdrew from the trial within the first 2 weeks of receiving the booster vaccine. We also assessed the outcomes of efficacy against first and multiple episodes of clinical malaria, according to primary and secondary case definitions, as well as efficacy against asymptomatic malaria.

Statistical analysis

Cox regression models were used to analyse time to first episodes of clinical malaria from 14 days following the booster vaccination to 12 months. For participants without an episode of clinical malaria, their time was censored at the date of their withdrawal from the study or the date of their 12-month post-booster blood sampling. The primary comparisons were prespecified as being between groups 1 and 3 and groups 2 and 3, with comparison of groups 1 and 2 combined with group 3 only considered if no significant difference was found between groups 1 and 2. A secondary analysis adjusted for confounding factors of sex, age at randomisation (categorised as 5–9 months, 10–12 months, and >12 months), and bednet use (adequate or not) during the malaria season. Vaccine efficacy was calculated as 1 minus the hazard ratio (HR).

	Group 1 (n=132)	Group 2 (n=137)	Group 3 (n=140)	Overall (n=409)
Mean age at first vaccine, months	11·4 (3·8)	11·3 (3·8)	12·1 (3·8)	11·6 (3·8)
Age category at first vaccine, months				
5–9	47 (36%)	48 (35%)	37 (26%)	132 (32%)
10–12	17 (13%)	21 (15%)	19 (14%)	57 (14%)
>12	68 (51%)	68 (50%)	84 (60%)	220 (54%)
Sex				
Male	59 (45%)	78 (57%)	64 (46%)	201 (49%)
Female	73 (55%)	59 (43%)	76 (54%)	208 (51%)
Indoor spraying, day 28*				
Yes	57 (43%)	62 (45%)	57 (41%)	176 (43%)
Data missing	1 (1%)	3 (2%)	..	4 (1%)
Adequate bed net use, day 28*				
Yes	123 (93%)	117 (85%)	125 (89%)	365 (89%)
Data missing	..	1 (1%)	..	1 (<1%)
Bednet use, day 28*				
Absent	1 (1%)	1 (1%)	3 (2%)	5 (1%)
ITN no holes	118 (89%)	112 (82%)	124 (89%)	354 (87%)
ITN with holes	13 (10%)	23 (17%)	13 (9%)	49 (12%)
Data missing	..	1 (1%)	..	1 (<1%)
At least one round† of SMC*				
Yes	127 (96%)	121 (88%)	131 (94%)	379 (93%)
Number of rounds† of SMC*				
0	5 (4%)	16 (12%)	9 (6%)	30 (7%)
1	9 (7%)	2 (1%)	7 (5%)	18 (4%)
2	33 (25%)	33 (24%)	30 (21%)	96 (23%)
3	48 (36%)	35 (26%)	49 (35%)	132 (32%)
4	36 (27%)	51 (37%)	44 (31%)	131 (32%)
5	1 (1%)	0	1 (1%)	2 (<1%)
Z score				
≥3SD and <2SD	28 (21%)	26 (19%)	31 (22%)	85 (21%)
≥2SD and <1SD	59 (45%)	50 (37%)	51 (36%)	160 (39%)
≥1SD and <median	33 (25%)	45 (33%)	37 (26%)	115 (28%)
≥median and <1SD	8 (6%)	15 (11%)	17 (12%)	40 (10%)
≥1SD and < 2SD	4 (3%)	1 (1%)	4 (3%)	9 (2%)

Data are n (%) or mean (SD); percentages do not always sum to 100 due to rounding. This table includes all participants who received the booster vaccination dose at 12 months following the primary series of vaccinations. Group 1 received 5 µg R21/25 µg Matrix-M, group 2 received 5 µg R21/50 µg Matrix-M, and group 3 (the control group) received the Rabivax-S rabies vaccine. ITN=insecticide-treated net. SMC=seasonal malaria chemoprevention. *28 days after first booster vaccination. †One round of seasonal malaria chemoprevention corresponds to three doses of treatment received per month.

Table 1: **Demographics and characteristics of participants**

	Number of participants with at least one episode of clinical malaria (%)	Unadjusted efficacy (95% CI)	p value	Adjusted efficacy* (95% CI)	p value
From 14 days to 12 months following booster vaccination					
Group 1					
Primary case definition	67/132 (51%)	71% (60–78)	<0·0001	70% (59–78)	<0·0001
Secondary case definition	72/132 (55%)	71% (61–78)	<0·0001	70% (60–78)	<0·0001
Group 2					
Primary case definition	54/137 (39%)	80% (72–85)	<0·0001	80% (72–85)	<0·0001
Secondary case definition	65/137 (47%)	77% (69–83)	<0·0001	77% (69–83)	<0·0001
Group 3 (control group)					
Primary case definition	121/140 (86%)	..	..	..	..
Secondary case definition	124/140 (89%)	..	..	..	..
From 14 days to 24 months following primary series of vaccinations					
Group 1					
Primary case definition	82/132 (62%)	66% (55–74)	<0·0001	66% (55–74)	<0·0001
Secondary case definition	89/132 (67%)	67% (56–75)	<0·0001	67% (56–75)	<0·0001
Group 2					
Primary case definition	70/137 (51%)	75% (66–81)	<0·0001	75% (66–81)	<0·0001
Secondary case definition	81/137 (59%)	75% (67–81)	<0·0001	75% (67–81)	<0·0001
Group 3 (control group)					
Primary case definition	128/140 (91%)	..	..	..	..
Secondary case definition	131/140 (94%)	..	..	..	..

Group 1 received 5 µg R21/25 µg Matrix-M, group 2 received 5 µg R21/50 µg Matrix-M, and group 3 received the Rabivax-S rabies vaccine. The primary case definition of clinical malaria in this study was presence of an axillary temperature 37·5°C or greater and *Plasmodium falciparum* parasite density greater than 5000 asexual forms per µL. The secondary case definition of clinical malaria was presence of an axillary temperature 37·5°C or greater or history of fever, or both, within the last 24 h and *P falciparum* parasite density greater than 0. The Cox proportional hazards model was used to calculate the hazard ratio (HR). Vaccine efficacy was calculated as 1–HR and expressed as a percentage. Further adjustment of efficacy took place for use of seasonal malaria chemoprevention (at least one monthly course of three doses) and this did not significantly change vaccine efficacy. *Cox proportional hazards model, adjusted for sex, age category (5–9 months, 10–12 months, and >12 months), and adequate insecticide-treated net use.

Table 2: **Time to first episode of malaria meeting case definitions of clinical malaria episode**

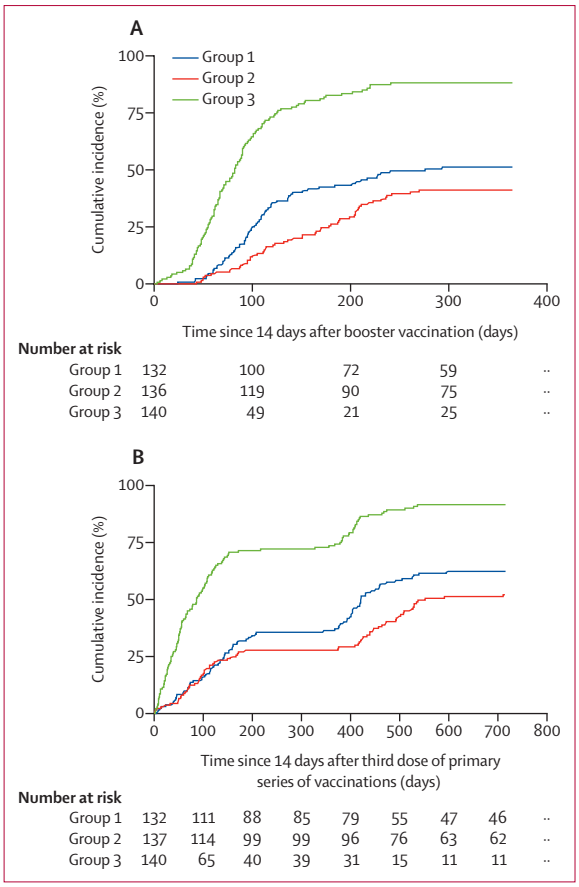

Figure 2: **Kaplan–Meier estimates of the time to first episode of clinical malaria according to the primary case definition**
The primary case definition of clinical malaria in this study was the presence of an axillary temperature of 37·5°C or greater and *Plasmodium falciparum* parasite density greater than 5000 asexual forms per µL. Analyses of vaccine efficacy included all participants who received a booster vaccination. (A) Data beginning from 14 days to 12 months after the booster vaccination. (B) Data beginning from 14 days to 24 months after the primary series of vaccinations. Group 1 received 5 µg R21/25 µg Matrix-M, group 2 received 5 µg R21/50 µg Matrix-M, and group 3, the control group, received the Rabivax-S rabies vaccine.

Analyses of vaccine efficacy included all participants who received a booster vaccination. Outcomes of asymptomatic malaria infection at 12 months following the booster vaccination were analysed by use of a log binomial model, including randomised group as a covariate. Relative risks and 95% CIs were reported, comparing groups 1 and 3 and groups 2 and 3.

Multiple episodes of the primary case definition were also analysed with negative binomial regression models, with follow-up time as an offset, unadjusted and adjusted for the same covariates as the primary analysis. To avoid double counting of episodes of the primary outcome that resulted in more than one health-care contact, attendances at clinics within 7 days of a previous episode were not counted. No adjustment was made to the person-time at risk after a disease episode.[7] To estimate the potential number of malaria cases averted by vaccination, the incidence rate differences per 1000 child-years at risk between the study groups were also calculated. This was done with a modified ordinary least squares regression approach, described previously,[8] which uses a robust standard error and controls for differences in individual person-time at risk.

To assess for an immunological correlate of protection, a Spearman's rank correlation between the number of malaria episodes and the anti-NANP immune response was done for groups 1 and 2 combined, and for each group and each year of follow-up separately. A linear regression model was used to estimate the difference in mean log(ELISA) between those with and without at least one episode of malaria. Additionally, HRs for log(ELISA) were calculated when included in the Cox model for time to first episode of malaria for the same groups and for each year. Finally, a reverse cumulative distribution of antibody titres was plotted and used to calculate a threshold level of anti-NANP antibodies for the efficacy observed in groups 1 and 2 in each year of follow-up.

	Number of total malaria episodes (%)	Unadjusted efficacy (95% CI)	p value	Adjusted efficacy* (95% CI)	p value	Rate difference per 1000 (95% CI)
Group 1						
12 months since booster	144/608 (24%)	61% (50–69)	<0·0001	60% (50–68)	<0·0001	1781 (1361–2200)
24 months since primary series	216/978 (22%)	63% (55–71)	<0·0001	63% (54–70)	<0·0001	1531 (1225–1836)
Group 2						
12 months since booster	83/608 (14%)	78% (71–83)	<0·0001	78% (71–83)	<0·0001	2285 (1911–2658)
24 months since primary series	141/978 (14%)	77% (70–82)	<0·0001	77% (70–82)	<0·0001	1853 (1561–2146)
Group 3 (control group)						
12 months since booster	381/608 (63%)	..	..	..	..	..
24 months since primary series	621/978 (64%)	..	..	..	..	..

Group 1 received 5 µg R21/25 µg Matrix-M, group 2 received 5 µg R21/50 µg Matrix-M, and group 3 received Rabivax-S. The primary case definition of clinical malaria was presence of axillary temperature 37·5°C or greater and *Plasmodium falciparum* parasite density greater 5000 asexual forms per µL. Attendance at clinic within 7 days of a previous episode was not counted. Negative binomial regression models were used to calculate vaccine efficacy when analysing multiple episodes. Wald test was used to calculate p value. Incidence rate differences were calculated per 1000 child-years at risk with ordinary least squares regression of transformed variables. *Protective efficacy adjusted for sex, age category (5–9 months, 10–12 months, and >12 months), and adequate insecticide-treated net use.

Table 3: **Analysis of multiple episodes of clinical malaria meeting the primary case definition from 14 days after the booster vaccination to 12 months, and from 14 days to 24 months following the primary series of vaccinations**

To facilitate blinding, analyses were done by statisticians external to the investigator teams.

All statistical analyses were done with Stata (version 16.1).

This study is registered with Clinicaltrials.gov (NCT03896724).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between May 7 and June 13, 2019, 450 participants were enrolled in the trial. 409 participants returned to receive the first booster vaccination in June 2–July 2, 2020, before the second malaria season (figure 1). 408 children were included in the time to first episode of malaria analyses (one was excluded due to withdrawal within 2 weeks of receiving the booster vaccine). Baseline demographic characteristics were similar across the three groups and participants were followed up for a median of 365 days (range 25–365) following the booster vaccination (table 1). There was no significant difference between baseline characteristics of children lost to follow-up and those still remaining in the trial (appendix 2 p 2).

Adequate use of insecticide-treated nets before the second malaria season was 89% (365 of 409) overall and indoor residual spraying was done in 176 (43%) of 409 households. 379 (93%) of 409 participants had at least one round of seasonal malaria chemoprevention (table 1).

According to the primary case definition, 242 participants had at least one episode of clinical malaria from 14 days to 12 months after the first booster vaccination. A Cox regression model comparing group 1, who received R21 with 25 µg Matrix-M, with the control group, resulted in an unadjusted vaccine efficacy of 71% (95% CI 60–78; p<0·0001). When comparing group 2, who received R21 with 50 µg Matrix-M, with the control group, the unadjusted vaccine efficacy was 80% (95% CI 72–85; p<0·0001; table 2, figure 2). There was a significant difference in efficacy between groups 1 and 2 (HR 0·68 [95% CI 0·48–0·98]; p=0·038); therefore, these groups were not combined in any efficacy analyses. In group 1, 50 (60%) of 84 participants who had no episodes of clinical malaria at 12 months following the primary series of vaccinations also had no episodes of clinical malaria at 12 months following the booster vaccination. This was the case for 68 (69%) of 99 participants in group 2 (appendix 2 p 3). 608 malaria episodes met the primary case definition in the 12 months' follow-up after the first booster vaccination; protective efficacy against multiple episodes of malaria was 61% (95% CI 50–69; p<0·0001) in group 1 and 78% (71–83; p<0·0001) in group 2 (table 3). The incidence rate difference, compared with the control group, indicated 1781 (95% CI 1361–2200) malaria cases averted per 1000 child-years at risk in group 1 and 2285 (1911–2658) malaria cases averted per 1000 child-years at risk in group 2 (table 3).

The primary analysis was repeated, adjusting for the potential confounding factors of sex, age at randomisation (5–9 months, 10–12 months, and >12 months), and adequate bednet use. Vaccine efficacy according to the primary case definition from 14 days following booster vaccination to 12 months was 70% (95% CI 59–78; p<0·0001) in group 1 and 80% (72–85; p<0·0001) in group 2 (table 2). Further adjustment for use of seasonal malaria chemoprevention (at least one monthly course of three doses) resulted in a vaccine efficacy of 81% (95% CI 74–87; p<0·0001) in group 2.

Efficacy was further assessed at 24 months (range 660–731 days) following the primary series of vaccinations, where 280 participants had at least one

	Year 1					Year 2				
	Number of cases	Mean, log ELISA (SD)	Difference in means* (95% CI); p value*	Spearman's ρ (95% CI); p value†	HR (95% CI); p value	Number of cases	Mean, log ELISA (SD)	Difference in means* (95% CI); p value*	Spearman's ρ (95% CI); p value†	HR‡ (95% CI); p value
At least one malaria case	88	3·8 (0·27)	0·14 (0·05 to 0·22); 0·001	–0·23 (–0·34 to –0·12); 0·0001	0·41 (0·22 to 0·75); 0·004	120	3·8 (0·46)	0·16 (0·05 to 0·27); 0·005	–0·27 (–0·37 to –0·17); <0·0001	0·73 (0·56 to 0·95); 0·017
No malaria cases	200	4·0 (0·35)	..	..	..	147	4·0 (0·44)	..	..	..

For year 1, antibody responses were measured by ELISA at 28 days following the primary series of vaccinations (three vaccinations, 4 weeks apart) and an episode of malaria according to the primary case definition from 28 days after the third vaccination to 12 months. For year 2, antibody responses were measured by ELISA at 28 days following the booster vaccination and an episode of malaria according to the primary case definition from 28 days after the booster vaccination to 12 months. *Calculated from a linear regression model. †p value for Spearman's rank correlation between number of malaria episodes and immune response. HR for log (ELISA) when included in Cox model for time to first episode of malaria in year 1 and in Cox model for year 2; group also included in model.

Table 4: **Correlation of NANP-specific IgG response data with malaria episodes for groups 1 and 2 combined**

episode of clinical malaria. All of these participants received a booster dose before the second malaria season, approximately 12 months following the primary series of vaccinations. These malaria episodes were recorded in 82 of 132 participants in group 1, 70 of 137 in group 2, and 128 of 140 participants in group 3 (the control group). Cox regression analysis showed a vaccine efficacy of 66% (95% CI 55–74; p<0·0001) for group 1 and 75% (66–81; p<0·0001) for group 2 (table 2, figure 1). When assessing multiple episodes of malaria over this time period, 978 cases were recorded and vaccine efficacy was similar to the analysis of a first or only event: 63% (95% CI 55–71; p<0·0001) in group 1 and 77% (69–83; p<0·0001) in group 2 (table 3).

Cross-sectional blood films were done at 12 months following the booster vaccination. In group 1, two (2%) of 122 children had asymptomatic parasitaemia, as did two (2%) of 125 in group 2. In group 3, seven (5%) of 129 children had asymptomatic parasitaemia (appendix 2 p 4). When compared with the control group (group 3), the risk ratios were not significant for group 1 (0·3 [95% CI 0·06–1·40]; p=0·125) or for group 2 (0·29 [0·06–1·39]; p=0·123).

Three serious adverse events were reported in participants (appendix 2 p 5) after the booster vaccination up to 12 months follow-up. All were deemed unrelated to vaccination. These serious adverse events all resolved and comprised severe malaria with pneumonia, severe malnutrition with anaemia, and bacterial meningitis.

At 28 days following the booster vaccination in the R21/Matrix-M malaria vaccine groups (groups 1 and 2), NANP IgG antibody concentrations were restored to levels similar to those observed following the primary series of vaccinations (appendix 2 p 7). The NANP IgG antibody concentrations at 28 days following the first booster vaccination were assessed for correlation with the number of clinical malaria episodes at 12 months following the booster vaccination. Across groups 1 and 2 combined, the difference in mean antibody titres between participants with or without at least one episode of malaria (calculated from a linear regression model) was 0·14 (95% CI 0·05 to 0·22; p=0·001) in the first year after three vaccinations and 0·16 (0·05 to 0·27; p=0·005) in the second year following the booster vaccination (table 4). Antibody concentrations correlated negatively with the number of malaria episodes for the first year (Spearman's ρ –0·23 [95% CI –0·34 to –0·12]; p=0·0001) and for the second year (–0·27 [–0·37 to –0·17], p<0·0001), and antibody titres had a significant effect on the time to first malaria episode in the first year (HR 0·41 [95% CI 0·22 to 0·75]; p=0·004) and the second year (0·73 [0·56 to 0·95]; p=0·017). When each vaccination group was assessed separately (appendix 2 p 6), similar significant correlations were observed for participants in group 2 for the first year (Spearman's ρ –0·32 [95% CI –0·45 to –0·19]; p=0·0001) and for the second year (–0·20 [–0·34 to –0·06]; p=0·02). Only the correlation between the number of malaria episodes and antibody concentrations over the second year of follow-up was significant (Spearman's ρ –0·11 [–0·28 to 0·07], p=0·210, for the first year and –0·28 [–0·44 to –0·12], p=0·0011, for the second year).

For participants in group 2, NANP antibodies were significantly higher in those who did not have any clinical malaria episodes during both the first year of follow-up (mean difference 0·21 [95% CI 0·10–0·33]; p=0·0004) and second year of follow-up (0·12 [0·01–0·22]; p=0·032). Cumulative distribution curves were used to identify a threshold correlate of vaccine efficacy for group 2 vaccinees: 6618 ELISA units (95% CI 5565–8397) in the first year of follow-up and 6130 ELISA units (5347–7179) in the second year of follow-up (appendix 2 p 8).

Discussion

We report high efficacy of the R21/Matrix-M malaria vaccine, which was maintained over 2 years of follow-up after the primary series of vaccinations, and 1 year following the booster vaccination. This study was done in an area of highly seasonal malaria transmission. These findings show that R21/Matrix-M has again reached the WHO-specified efficacy goal of 75% or greater over 24 months in the target population of African children.[5] In children who received R21 with the higher dose of Matrix-M adjuvant, efficacy was 80% at 12 months following the booster vaccination.

A larger number of clinical malaria cases meeting the primary case definition were noted in the second year, 12 months after the booster vaccination. Variability between malaria seasons is expected and this was consistent with local malaria prevalence data.[9]

Induction of high concentrations of antibodies required to provide protection has been very difficult in malaria, and when attained, antibody titres decline rapidly.[4] We show that a single booster dose of R21/Matrix-M can restore high antibody concentrations. Administration of this booster dose led to sustained protective immunity over the second year when administering R21 with the higher adjuvant dose. Both the level of protective efficacy and its maintenance for a second year are promising for the potential utility of this malaria vaccine candidate. It is unclear whether a further dose will be required to maintain the high efficacy observed; the current trial has been extended for a further 2 years to assess the value of additional annual booster vaccine doses.

For vaccines targeting a range of pathogens, identification of immune correlates of protection has been important in understanding the protective immunity induced. Correlates enable assessment of potential efficacy in a range of populations without additional efficacy trials, as well as potentially supporting regulatory approvals. Most malaria vaccine candidates targeting the *P falciparum* circumsporozoite protein have aimed to induce protective antibodies to the highly conserved NANP repeat sequence. In trials of both the RTS,S/AS01 malaria vaccine and of this R21/Matrix-M vaccine candidate, these NANP antibodies have correlated with protection in several challenge studies in non-immune adults.[10,11] In the phase 3 trial evaluating the RTS,S/AS01 vaccine, these antibodies correlated with vaccine efficacy in young infants (aged 6–12 weeks), but not in children aged 5–17 months, the current target population for this vaccine.[4] In both the first and second year of follow-up, when assessing protection against the first or any malaria episode, we found that anti-NANP concentrations correlated significantly with vaccine efficacy in those who received R21/Matrix-M with the higher adjuvant dose (group 2). Analysis of the reverse cumulative distribution data suggests that a level of more than 6500 ELISA units per mL in these participants was associated with a 77% reduction in the risk of malaria. This correlate will be assessed further in future trials but might provide a useful biomarker of protection in African children.

In group 1 participants, who received R21/Matrix-M with a lower dose of adjuvant, antibody concentrations were almost halved at 28 days following the primary series of vaccinations when compared with participants in group 2 who received double the adjuvant dose.[6] A lower efficacy was observed in group 1 compared with group 2 participants in the first and second year of follow-up. After 2 years, vaccine efficacy in group 1 dropped to 70% (95% CI 59–78). This was significantly lower than in group 2, where vaccine efficacy was 80% (95% CI 72–85; p<0·0001), consistent with lower vaccine immunogenicity in group 1.

Limitations of this study include the small sample size, which restricts identification of less common adverse events, and reduces the power to identify a potential correlate of protection. Furthermore, this study assessed the efficacy of the R21/Matrix-M vaccine in an area of highly seasonal malaria transmission, with vaccines administered before or at the start of the peak malaria season. Data are needed on vaccine efficacy in areas with different transmission patterns.

Delivered seasonally, the R21/Matrix-M vaccine continues to show an acceptable safety profile in the second year of follow-up and following a fourth dose. Together with maintained high efficacy, these findings suggest that this malaria vaccine and vaccination strategy could have a substantial impact in areas of highly seasonal malaria transmission in Africa. These areas account for about half of all childhood deaths from malaria.[12] A potential correlate of protection has been identified and future work will continue to evaluate immune correlates in other settings in west and east Africa. R21/Matrix-M has now progressed to a phase 3 licensure trial. This trial is now fully enrolled and evaluating vaccine safety and efficacy in 4800 children at five sites in east and west Africa, including sites with perennial malaria transmission.

Contributors

AVSH, HT, and MSD conceived and designed the trial, and AVSH was the chief investigator. AVSH, HT, MSD, KJE, HMN, HS, and AS contributed to the protocol and design of the study. HT and HS were the study site principal investigators. KJE and DB were responsible for laboratory studies of immune responses and assay development. NW, MC, and KJE did the statistical analysis. US, GG, and LF were responsible for vaccine and adjuvant manufacturing and provision. MSD, HMN, AVSH, and HT contributed to the preparation of the report. HMN, HT, HS, AS, FO, TR, MSD, AVSH, KJE, and RR contributed to the implementation of the study. HMN, HT, TR, SS, NW, MC, MSD, and AVSH have accessed and verified the data. HMN, MSD, AVSH, and HT were responsible for the decision to submit the manuscript for publication. All other authors contributed to the implementation of the study and data collection. All authors critically reviewed and approved the final version. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

AVSH and KJE are named as co-inventors on patent applications related to R21. GG, LF, and JR are employees of Novavax, developers of the Matrix-M adjuvant. US is an employee of the Serum Institute of India, a co-developer of the R21/Matrix-M vaccine. All other authors declare no competing interests.

Data sharing

The study protocol is provided in appendix 2 (pp 9–105). Anonymised participant data will be made available when the trial is complete, upon requests directed to the corresponding authors. Proposals will be reviewed and approved by the sponsor, investigators, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. All data will be made available for a minimum of 5 years from the end of the trial.

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