# Safety and efficacy of the blood-stage malaria vaccine RH5.1/Matrix-M in Burkina Faso: interim results of a double-blind, randomised, controlled, phase 2b trial in children

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## Summary

**Background** Two pre-erythrocytic vaccines (R21/Matrix-M and RTS,S/AS01) are now approved for *Plasmodium falciparum* malaria. However, neither induces blood-stage immunity against parasites that break through from the liver. RH5.1/Matrix-M, a blood-stage *P falciparum* malaria vaccine candidate, was highly immunogenic in Tanzanian adults and children. We therefore assessed the safety and efficacy of RH5.1/Matrix-M in Burkinabe children.

**Methods** In this double-blind, randomised, controlled, phase 2b trial, RH5.1/Matrix-M was given to children aged 5–17 months in Nanoro, Burkina Faso, a seasonal malaria transmission setting. Children received either three intramuscular vaccinations with 10 µg RH5.1 protein with 50 µg Matrix-M adjuvant or three doses of rabies control vaccine, Rabivax-S, given either in a delayed third-dose (0, 1, and 5 month) regimen (first cohort) or a 0, 1, and 2 month regimen (second cohort). Vaccinations were completed part way through the malaria season. Children were randomly assigned 2:1 within each cohort to receive RH5.1/Matrix-M or Rabivax-S. Participants were assigned according to a random allocation list generated by an independent statistician using block randomisation with variable block sizes. Participants, their families, and the study teams were masked to group allocation; only pharmacists who prepared the vaccines were unmasked. Vaccine safety, immunogenicity, and efficacy were evaluated. The coprimary outcomes assessed were: first, the safety and reactogenicity of RH5.1/Matrix-M; and second, the protective efficacy of RH5.1/Matrix-M against clinical malaria (measured as time to first episode of clinical malaria, using a Cox regression model) from 14 days to 6 months after the third vaccination in the per-protocol sample. This ongoing trial is registered with ClinicalTrials.gov (NCT05790889).

**Findings** From April 6 to 13 and July 3 to 7, 2023, 412 children aged 5–17 months were screened, and 51 were excluded. A total of 361 children were enrolled in this study. In the first cohort, 119 were assigned to the RH5.1/Matrix-M delayed third-dose group, and 62 to the equivalent rabies control group. The second cohort included 120 children in the monthly RH5.1/Matrix-M group and 60 in the equivalent rabies control group. The final vaccination was administered to all groups from Sept 4 to 21, 2023. RH5.1/Matrix-M in both cohorts had a favourable safety profile and was well tolerated. Most adverse events were mild, with the most common being local swelling and fever. No serious adverse events were reported. Comparing the RH5.1/Matrix-M delayed third-dose regimen with the pooled control groups resulted in a vaccine efficacy of 55% (95% CI 20 to 75%; p=0·0071). The same analysis showed a vaccine efficacy of 40% (–3 to 65%; p=0·066) when comparing the monthly regimen with the pooled control groups. Participants vaccinated with RH5.1/Matrix-M in both cohorts showed high concentrations of anti-RH5.1 serum IgG antibodies 14 days after the third vaccination, and the purified IgG showed high levels of in vitro growth inhibition activity against *P falciparum*; these responses were higher in patients who received the RH5.1/Matrix-M vaccine delayed third-dose regimen, as opposed to monthly regimen (growth inhibition activity 79·0% [SD 14·3] *vs* 74·2% [SD 15·9]; p=0·016).

**Interpretation** RH5.1/Matrix-M appears safe and highly immunogenic in African children and shows promising efficacy against clinical malaria when given in a delayed third-dose regimen. This trial is ongoing to further monitor efficacy over time.

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## Introduction

Malaria caused by the *Plasmodium falciparum* parasite continues to exert a heavy disease burden across sub-Saharan Africa.[1] However, two first-generation, partly effective pre-erythrocytic vaccines (RTS,S/AS01 and R21/Matrix-M) are now recommended for malaria prevention in children using a four-dose schedule from approximately the age of 5 months. These two vaccines are similar in design and target the liver-invasive sporozoite.[2,3] However, when this immunity fails or wanes over time and sporozoites infect the liver, blood-stage infection ensues with a risk of clinical disease. Vaccination against the blood-stage merozoite would thus provide a second line of defence. However, the development of an effective blood-stage vaccine has proved challenging,[4] with all previous phase 2b field efficacy trials reporting either no or minimal efficacy, or only evidence of strain-specific efficacy linked to target antigen polymorphism.[5–9]

Identification of the reticulocyte-binding protein homologue 5 (RH5) as a vaccine target[10] has since transformed the blood-stage *P falciparum* vaccine field. This merozoite protein forms an essential interaction with basigin (CD147) on the human red blood cell during invasion[11] and, unlike previous antigen targets, is almost completely conserved, probably explaining the human species tropism of this parasite.[12] We have previously shown the high-level efficacy of RH5-based vaccination in non-human primates[13] and a significant reduction in the parasite growth rate in UK adult vaccinees after controlled human malaria infection.[14] In both the non-human primate and human studies we observed a strong correlation between the in vivo reduction of parasite growth and in vitro growth inhibition activity (GIA),[13,14] since validated as a mechanistic immune correlate in non-human primates.[15] Human anti-RH5 monoclonal antibodies isolated from vaccinees have also shown promising in vitro GIA against laboratory-adapted parasite lines and west African clinical parasite isolates.[16,17] We have also reported promising safety and reactogenicity data from four phase 1a/b trials of RH5-based vaccine

### Research in context

#### Evidence before this study

Two partly effective pre-erythrocytic vaccines (RTS,S/AS01 and R21/Matrix-M) using a four-dose schedule are now approved for malaria prevention in children from the age of approximately 5 months. Both vaccines induce antibodies that prevent liver infection by the *Plasmodium falciparum* parasite; however, because this pre-erythrocytic immunity fails or wanes over time, parasites can break through into the blood, leading to clinical disease. Given there are no licensed vaccines to protect against the blood stage of *P falciparum*, there is currently no immunological second line of defence.

We searched PubMed on Oct 14, 2024, from database inception for research articles using the terms: "malaria vaccine" AND "blood stage" AND ("phase 1–2b" OR "phase II"). No time or language filters were applied. This search identified field efficacy trial results in children for five blood-stage vaccine candidates: combination B, FMP1/AS02, AMA1-C1/Alhydrogel, FMP2.1/AS02$_A$, and GMZ2/Alum. All these candidate vaccines reported either no or minimal efficacy or evidence of strain-specific efficacy linked to target antigen polymorphism. The more recent report in 2011 of the identification of the highly conserved, essential, and antibody-susceptible RH5 blood-stage vaccine antigen has reinvigorated the field of blood-stage vaccines. However, no RH5-based vaccine has been tested for efficacy against clinical malaria in children. Having shown promising immunogenicity of the RH5.1 protein vaccine candidate adjuvanted with Matrix-M in Tanzanian children, we set out to address this unmet need by assessing the safety and efficacy of RH5.1/Matrix-M in a seasonal malaria setting in Burkinabe children.

#### Added value of this study

This study reports the efficacy of the blood-stage malaria vaccine candidate RH5.1/Matrix-M in a phase 2b trial in children living in a malaria-endemic setting with seasonal transmission in Burkina Faso. We show that this vaccine candidate, administered during the malaria transmission season, has promising efficacy against clinical malaria over the first 6 months of follow-up when delivered in a 0, 1, and 5 month (delayed third-dose) regimen as opposed to a 0, 1, and 2 month schedule. Furthermore, the RH5.1/Matrix-M vaccine has a favourable safety profile; it is well tolerated, with most local and systemic adverse events graded as mild, and no serious adverse events reported. Vaccination in three-dose regimens resulted in high levels of malaria-specific anti-RH5.1 antibodies, which showed functional in vitro growth inhibition activity. This phase 2b trial is currently continuing into a second malaria season, including a fourth (ie, booster) dose of RH5.1/Matrix-M at 12 months, to further measure vaccine efficacy over time.

#### Implications of all the available evidence

These data suggest the importance of a delayed third-dose regimen in maximising the efficacy of a blood-stage vaccine against clinical malaria and strongly support the future evaluation of RH5.1/Matrix-M in combination with one of the licensed pre-erythrocytic vaccines as a multi-stage malaria vaccine strategy. Such future trials should include different malaria transmission settings, including those with higher and perennial malaria transmission. A combination of pre-erythrocytic and blood-stage vaccines could function additively, or even synergistically, and this is now seen as the most promising strategy to the rapid registration of a second-generation paediatric malaria vaccine with high and durable efficacy.

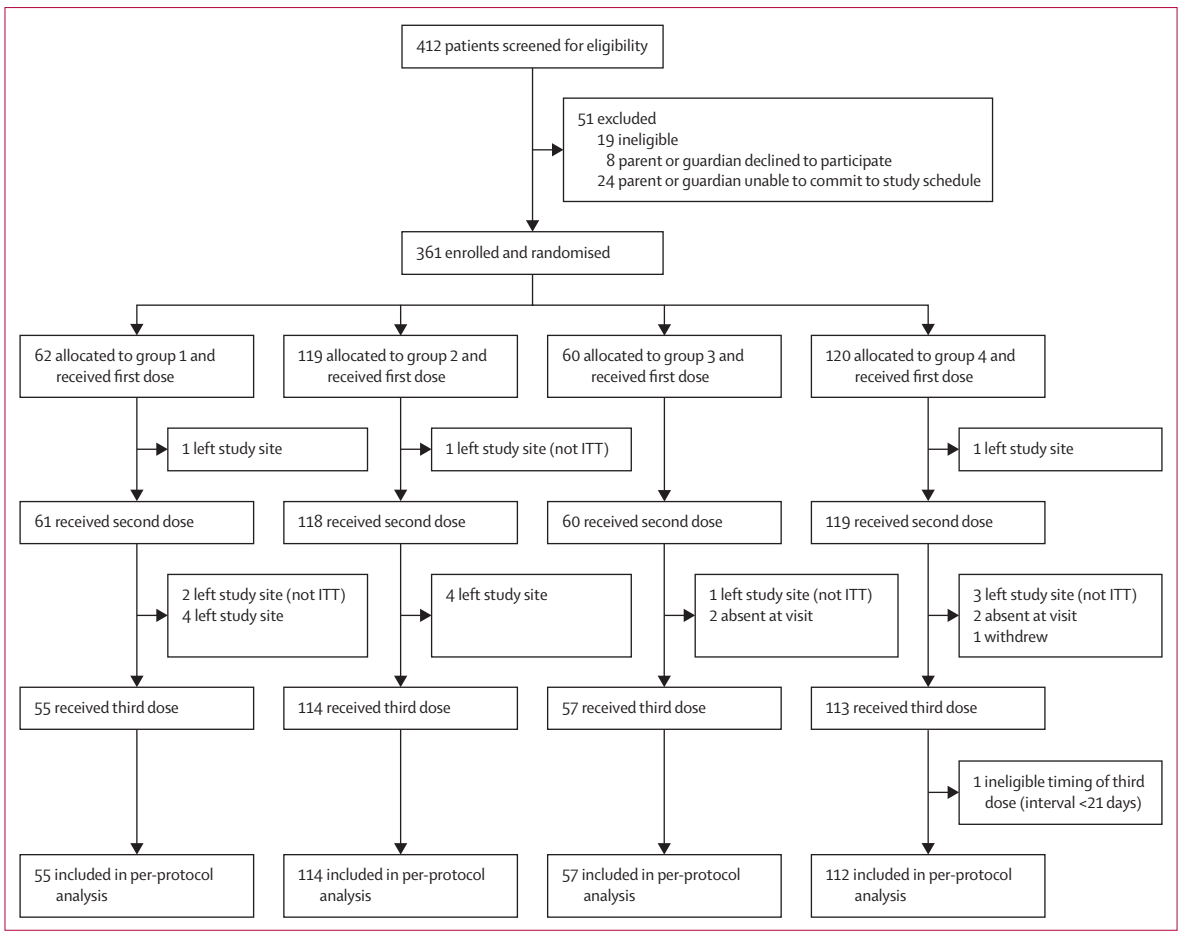


***Figure 1:*** **Trial profile**
Participants allocated to the delayed regimen received vaccinations at 0, 1, and 5 months. Participants allocated to the monthly regimen received vaccinations at 0, 1, and 2 months. Participants were aged 5–17 months at enrolment (first day of vaccination). Per-protocol analysis included all participants who received three doses and were analysed at 6 months. Group 1 received the rabies vaccine (Rabivax-S) with a delayed third dose. Group 2 received the RH5.1/Matrix-M vaccine with a delayed third dose. Group 3 received the rabies vaccine (Rabivax-S) in a monthly regimen. Group 4 received the RH5.1/Matrix-M vaccine in a monthly regimen. The primary safety analysis used the ITT sample of any child who received at least the first dose of vaccine (N=361) and the primary efficacy analysis used the per-protocol sample (N=338). The secondary efficacy analyses excluded seven children from the ITT sample (indicated in the figure) who left the study site for a follow-up period starting at least 14 days after their last dose of vaccine (N=354). ITT=intention-to-treat.

candidates in 193 adults, children, and infants in the UK and Tanzania.[14,18–20] Notably, the RH5.1 protein[21] with Matrix-M adjuvant in Tanzanian children aged 5–17 months resulted in the highest human vaccine-induced GIA ever reported, exceeding the protective threshold identified in the non-human primate model.[13,20] We therefore initiated a phase 2b trial (called VAC091) of this vaccine candidate in children aged 5–17 months in Nanoro, an area with seasonal malaria transmission in Burkina Faso, to assess the protective efficacy of this vaccine candidate against clinical malaria.

## Methods

### Study design and participants

VAC091 is a double-blind, randomised, controlled, phase 2b trial conducted by the Institut de Recherche en Sciences de la Santé at the Clinical Research Unit of Nanoro (Nanoro, Burkina Faso) and sponsored by the University of Oxford (Oxford, UK). Participants aged 5–17 months were recruited at the Siglé trial site, located within the Nanoro Health and Demographic Surveillance System catchment area. This area covers 24 villages, with a population of more than 63 000 inhabitants. Nanoro is an area where malaria transmission occurs throughout the year, but with a marked peak during the rainy season (June to November).

Eligible participants were recruited into four groups (figure 1; appendix p 24). Groups 1 and 2 (first cohort) received the delayed third-dose (a dose at 0, 1, and 5 months) regimen (hereafter referred to as the delayed regimen) of rabies or RH5.1/Matrix-M vaccines for group 1 and group 2, respectively. Groups 3 and 4 (second cohort) received these vaccines in a monthly regimen (0, 1, and 2 months). The third vaccination was given

See Online for appendix

simultaneously across all four groups and completed part way through the malaria season. Field workers collected data on indoor residual spraying of households, insecticide-treated net use and if the nets were adequate (based on the presence of holes), and the number of doses and months of seasonal malaria chemoprevention taken by the participant during the malaria transmission season.

After community sensitisation, a list of eligible children was drawn from the Nanoro Health and Demographic Surveillance System database, and parents or legally authorised guardians who expressed interest were invited to screening visits. Before recruitment, parents or guardians of participants provided written or thumb-printed consent, which was verbally checked at each study visit. Inclusion criteria specified that participants should be aged 5–17 months at enrolment and aimed to be living in the study area for the whole trial duration. Exclusion criteria included significant comorbidities and participation in other malaria intervention studies and clinical trials. Further details are given in the appendix (pp 8–9).

The trial was approved by the National Ethical Committee of the Ministry of Health (Comité d'Ethique pour la Recherche en Santé, reference number 2022–12–256), and the national regulatory authority in Burkina Faso, Agence National de Régulation Pharmaceutique (reference number 2023/0208/MSHP/SG/ANRP/DHEC/MIK). Ethical approval was granted in the UK by the Oxford Tropical Research Ethics Committee (reference number 3–23). This study is registered with ClinicalTrials.gov (NCT05790889).

### Randomisation and masking

Children aged 5–17 months were randomly assigned (1:2) to groups 1 and 2 (delayed regimen) in the first cohort and similarly to groups 3 and 4 (monthly regimen) in the second cohort. A statistician generated a random allocation list, using block randomisation with variable block sizes, and prepared and sealed the envelopes using this list, which was then given to the pharmacist to assign to participants. Both malaria and control vaccines were prepared by the pharmacist using the same type of syringe, and the contents of the syringe were covered with an opaque label. The trial was double-masked; participants, their families, the central and local study teams, and laboratory teams were all masked to group allocation. Only the pharmacists preparing the vaccines and statisticians were unmasked to group allocation. No unmasking of study investigators or participants occurred during the study.

### Procedures

The RH5.1 soluble protein was originally produced according to Good Manufacturing Practice by the Clinical Biomanufacturing Facility in Oxford, UK.[21] A second batch was filled in 2021 under Good Manufacturing Practice by a Contract Manufacturing Organisation in the UK and this batch was used in this trial. A 10 µg dose of RH5.1 protein was mixed with 50 µg Matrix-M adjuvant immediately before administration. Matrix-M is a potent, saponin-based adjuvant and (for the batch used in this trial) was manufactured jointly by Novavax AB (Uppsala, Sweden) and the Serum Institute of India (Pune, India). A rabies vaccine (Rabivax-S), manufactured by the Serum Institute of India, was the control vaccine. All vaccines were administered intramuscularly into the thigh.

On the day of enrolment, a blood film was performed to check for *Plasmodium* spp parasites. In the absence of a fever of 37·5°C or higher, or a history of fever within the last 24 h, or both, participants proceeded to vaccination, but if they were then film positive, they received treatment for malaria in accordance with national guidelines. For each subsequent vaccination, participants were tested for malaria if they had a fever of 37·5°C or higher, or a history of fever within the last 24 h, or both. If their blood film was positive, they were treated for malaria before being vaccinated upon recovery (usually on day 4, after completing 3 days of treatment, and after a repeat negative blood film). After each vaccination, local and systemic solicited adverse events were collected for 7 days. Unsolicited adverse events were collected for 28 days after vaccination and classified according to MedDRA (version 27.0). Severity and causality of adverse events were assessed using standardised methods (appendix pp 10–12, 25–26) and followed up until resolution. Safety laboratory values were measured at 14 days after the second vaccination, the day of third vaccination (groups 1 and 2 only), and 2 months and 6 months after the third vaccination to look for deviations from baseline. Serious adverse events were recorded for the whole duration of the study. A data safety monitoring board review was held after the first vaccination of the first 100 participants.

Malaria case detection was by a mixture of passive and active detection methods. Parents of participants were advised to attend the trial site or community health centres if their child had any illness or fever, for review and assessment for malaria. After the third vaccination, participants were also visited by field workers approximately every 30 days up to 6 months after the third vaccination, and a blood spot was taken at each visit for parasite quantification and genotyping. If the participants had a temperature of 37·5°C or higher or a history of fever within the last 24 h, or both, blood sampling was also performed for blood film microscopy to detect *Plasmodium* spp.

Anti-RH5.1 serum total IgG responses were measured by ELISA against full-length RH5 protein (RH5.1), using standardised methods as previously described.[20] Standardised GIA assays were performed by the GIA Reference Center (National Institutes of Health, MD, USA) using previously described methods[22] (appendix pp 22–23).

### Outcomes

The coprimary outcomes assessed were: first, the safety and reactogenicity of RH5.1/Matrix-M, and second, the protective efficacy of RH5.1/Matrix-M against clinical malaria from 14 days to 6 months after the third vaccination. The primary case definition of clinical malaria was the presence of an axillary temperature of 37·5°C or higher, or a history of fever in the past 24 h, or both, and *P falciparum* asexual parasite density of more than 5000 parasites per µL, as measured by blood film microscopy. Secondary case definitions were the presence of an axillary temperature of 37·5°C or higher, or a history of fever during the last 24 h, or both; and *P falciparum* parasite density of more than 0 parasites per µL or a parasite density of more than 20 000 parasites per µL, as measured by blood film microscopy. Post-hoc analyses assessed additional case definitions with parasite densities of more than 50 000 parasites per µL and more than 100 000 parasites per µL. The secondary outcomes assessed were: first, the protective efficacy of RH5.1/Matrix-M against clinical malaria from 14 days to 3 months after the third vaccination; second, the protective efficacy of RH5.1/Matrix-M against prevalent moderate or severe anaemia at 6 months after the third vaccination; and third, the humoral immunogenicity of RH5.1/Matrix-M. Analysis of other prespecified secondary outcomes regarding the primary vaccination series is in progress and will be reported after the end of the trial (appendix pp 12–15).

### Statistical analysis

It was estimated that 104 children per group would give 90% power to detect a 50% vaccine efficacy in either group 2 or 4 compared with the pooled controls (groups 1 and 3) if there were 1·2 episodes of clinical malaria per child in the first 6 months of follow-up in the control group (appendix p 7). The rate of 1·2 events per child came from a study previously conducted in the same area.[3] 120 children per group were recruited to allow for a 15% loss to follow-up.

For the coprimary endpoint of vaccine safety and reactogenicity, odds ratios (ORs) comparing the proportion of doses that resulted in solicited adverse events were calculated using logistic regression. This analysis used the intention-to-treat sample of any child who received at least the first dose of vaccine (N=361). For the coprimary endpoint of vaccine efficacy, Cox regression models were used to analyse the time to the first episode of clinical malaria (as per the primary case definition) within 6 months after the third vaccination. Follow-up time started 14 days after the third vaccination. Vaccine efficacy was calculated as 1 minus the hazard ratio. The secondary and additional case definitions of clinical malaria were analysed in the same way. A secondary analysis of vaccine efficacy against all clinical malaria episodes was also done, using Cox regression models with a robust standard error to account for multiple episodes. Episodes occurring within 14 days of a previous episode were classed as the same event. For participants without an episode of clinical malaria, their time was censored at the date of their withdrawal or the date of their 6-month blood sampling (noting no deaths had occurred in this trial). The proportional hazards assumption of the Cox model was checked by inspecting plots of log(-log[S(t|x)]) against log(t) and of Schoenfeld residuals. Neither of these plots showed notable deviations from the proportional hazards assumption. A secondary analysis of the time to the first episode of clinical malaria (analysed as per the primary endpoint) but restricted to episodes occurring within 3 months of the third vaccination was also done (appendix pp 43–45). The primary comparisons were prespecified as being between group 2 and the pooled control groups 1 and 3, and between group 4 and the pooled groups 1 and 3, with comparison between groups 1 and 2 and between groups 3 and 4 as a supplementary analysis. A secondary analysis adjusted for confounding factors including total number of rounds of seasonal malaria chemoprevention received, insecticide-treated net use (adequate or not) the night before the screening visit, and age at random assignment (5–8 months, 9–12 months, or 13–17 months). Event rates of malaria are also reported here, but for information only.

The primary analysis of vaccine efficacy was based on a per-protocol sample, which included all participants who received three vaccinations correctly and within the prespecified time period (N=338). Secondary analyses of vaccine efficacy included the intention-to-treat sample of any child who received at least the first dose of vaccine and remained in the site for a follow-up period starting at least 14 days after their last dose of vaccine (N=354).

Assays to measure in vitro *P falciparum* GIA and serum anti-RH5.1 IgG responses were conducted on blood samples taken at baseline (screening) and at day 14 after third vaccination. GIA data were expressed as percentages and compared between the combined control groups and each RH5.1/Matrix-M vaccine group, and between the two RH5.1/Matrix-M vaccine groups. The measure of effect was the difference in mean percentage GIA, with inference done using the bootstrap method. Serum anti-RH5.1 IgG concentrations measured by ELISA were log10-transformed and the same between-group comparisons were performed by linear regression. All statistical analyses were performed by independent statisticians using Stata (version 18).

### 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

From April 6 to 13, 2023, 208 children aged 5–17 months were screened, and from July 3 to 7, 2023, a further

| | Overall (N=338) | Group 1: delayed rabies (n=55) | Group 2: delayed RH5.1/ Matrix-M (n=114) | Group 3: monthly rabies (n=57) | Group 4: monthly RH5.1/ Matrix-M (n=112) |
|---|---|---|---|---|---|
| Age, months | 10·5 (3·6) | 10·5 (3·7) | 10·6 (3·6) | 10·3 (3·6) | 10·5 (3·5) |
| Age category | | | | | |
| 5–8 months | 117 (35%) | 19 (35%) | 36 (32%) | 21 (37%) | 41 (37%) |
| 9–12 months | 92 (27%) | 16 (29%) | 35 (31%) | 13 (23%) | 28 (25%) |
| 13–17 months | 129 (38%) | 20 (36%) | 43 (38%) | 23 (40%) | 43 (38%) |
| Sex | | | | | |
| Female | 177 (52%) | 29 (53%) | 59 (52%) | 29 (51%) | 60 (54%) |
| Male | 161 (48%) | 26 (47%) | 55 (48%) | 28 (49%) | 52 (46%) |
| Insecticide-treated net* use the night before | | | | | |
| Yes: adequate | 290 (86%) | 43 (78%) | 86 (75%) | 52 (91%) | 109 (97%) |
| Yes: insecticide-treated net damaged | 7 (2%) | 1 (2%) | 6 (5%) | 0 | 0 |
| No: has insecticide-treated net | 27 (8%) | 9 (16%) | 14 (12%) | 3 (5%) | 1 (1%) |
| No: no insecticide-treated net | 14 (4%) | 2 (4%) | 8 (7%) | 2 (4%) | 2 (2%) |
| Indoor residual spraying with insecticide* in the past year | 8 (2%) | 4 (7%) | 4 (4%) | 0 | 0 |
| Seasonal malaria chemoprevention* coverage | | | | | |
| At least 1 round | 333 (99%) | 54 (98%) | 111 (97%) | 57 (100%) | 111 (99%) |
| 1 round | 33 (10%) | 5 (9%) | 23 (20%) | 2 (4%) | 3 (3%) |
| 2 rounds | 72 (21%) | 13 (24%) | 28 (25%) | 12 (21%) | 19 (17%) |
| 3 rounds | 116 (34%) | 23 (42%) | 27 (24%) | 20 (35%) | 46 (41%) |
| 4 rounds | 112 (33%) | 13 (24%) | 33 (29%) | 23 (40%) | 43 (38%) |
| Missing seasonal malaria chemoprevention info | 5 (1%) | 1 (2%) | 3 (3%) | 0 | 1 (1%) |
| Weight for age Z scores | | | | | |
| –3 to –2 SD | 38 (11%) | 8 (15%) | 18 (16%) | 6 (11%) | 6 (5%) |
| –2 to –1 SD | 94 (28%) | 13 (24%) | 29 (25%) | 14 (25%) | 38 (34%) |
| –1 SD to median | 141 (42%) | 21 (38%) | 46 (40%) | 27 (47%) | 47 (42%) |
| Median to +1 SD | 51 (15%) | 12 (22%) | 15 (13%) | 8 (14%) | 16 (14%) |
| +1 to +2 SD | 13 (4%) | 1 (2%) | 6 (5%) | 1 (2%) | 5 (4%) |
| +2 to +3 SD | 1 (<1%) | 0 | 0 | 1 (2%) | 0 |

Data are n (%) or mean (SD). Table includes 338 trial children who received all three eligible doses of RH5.1/Matrix-M or Rabivax-S (rabies) vaccine. *In 2023 (up to four monthly rounds of seasonal malaria chemoprevention).

***Table 1:*** **Comparability of trial sample at screening**

204 children aged 5–17 months were screened (figure 1). 51 were excluded and 361 were enrolled across two cohorts. In the first cohort, 119 children were allocated to receive RH5.1/Matrix-M in a delayed third-dose regimen (group 2) and 62 children were allocated to rabies vaccination in the same regimen (group 1). In the second cohort, 120 children were allocated to RH5.1/Matrix-M in a monthly regimen (group 4) and 60 children were allocated to receive rabies vaccination in the same regimen (group 3). The final vaccination of the primary series was administered contemporaneously across both cohorts from Sept 4 to 21, 2023, in the middle of the malaria season. 22 participants received fewer than three vaccinations, and one participant in group 3 who received three vaccinations had an interval of less than 21 days between the second and third vaccine doses, so 338 participants were included in the per-protocol analysis at 6 months after the third vaccination.

Baseline characteristics were similar across the four study groups, with an overall mean age at screening of 10·5 months (SD 3·6), and 177 (52%) of the enrolled participants being female (table 1). Of the 338 participants, 290 (86%) slept under an adequate insecticide-treated net the night before screening, and 333 (99%) received at least one round of seasonal malaria chemoprevention. However, only eight (2%) of 338 participants lived in a house that had received indoor residual spraying with insecticide in the past year.

Analysis of the primary endpoint of the time to the first episode of clinical malaria (as per the primary case definition) within 14 days to 6 months after the third vaccination, using a Cox regression model to compare the RH5.1/Matrix-M delayed third-dose regimen with the pooled control groups, resulted in a vaccine efficacy of 55% (95% CI 20 to 75%; p=0·0071); the same analysis showed a vaccine efficacy of 40% (–3 to 65%; p=0·066) when comparing the monthly regimen with the pooled control groups (table 2; figure 2A).

During this primary objective study period (within 14 days to 6 months after the third vaccination) a first episode of clinical malaria occurred in 17 of 114 participants (15%) in the delayed RH5.1/Matrix-M group (event rate 0·09/100 child days); 22 of 112 participants (20%) in the monthly RH5.1/Matrix-M group (event rate 0·12/100 child-days); and 33 of 112 participants (29%) in the pooled rabies control groups (event rate 0·21/100 child days; table 2). In total, 72 of 338 participants (21%) had at least one episode of clinical malaria according to the primary case definition; however, of these, ten participants also had more than one episode. In a secondary analysis, vaccine efficacy against all clinical malaria episodes up to 6 months (as per the primary case definition), analysed using Cox regression models, was 56% (95% CI 24–74%; p=0·0035) for the delayed and 40% (1–64%; p=0·045) for the monthly RH5.1/Matrix-M regimen (appendix pp 33–34).

As a secondary objective, we also analysed the time to the first episode of clinical malaria (as per the primary case definition) within 14 days to 3 months after the third vaccination using a Cox regression model. Here, a vaccine efficacy of 56% (95% CI 21–76%; p=0·0062) in the delayed regimen and 52% (13–73%; p=0·015) in the monthly regimen was observed when comparing with the pooled control groups (appendix pp 43–44). Here, 65 of 338 participants (19%) had at least one episode of malaria according to the primary case definition during this period.

We also analysed the time to the first episode of clinical malaria, using a Cox regression model, according to the secondary case definitions of clinical malaria during the 6-month period with a parasitaemia of more than 0 per µL; here, a vaccine efficacy of 44% (95% CI 8 to 66%;

| | N | Event rate per 100 days* | Vaccine efficacy (95% CI) | p value | Adjusted vaccine efficacy (95% CI) | Adjusted p value |
|---|---|---|---|---|---|---|
| **Primary case definition (parasitaemia >5000 parasites per µL)** | | | | | | |
| Rabies controls (combined) | 112 | 0·21 (33/156·3) | 1 | .. | 1 | .. |
| Delayed RH5.1/Matrix-M | 114 | 0·09 (17/185·0) | 0·55 (0·20 to 0·75) | 0·0071 | 0·57 (0·23 to 0·77) | 0·0051 |
| Monthly RH5.1/Matrix-M | 112 | 0·12 (22/176·4) | 0·40 (–0·03 to 0·65) | 0·0657 | 0·42 (–0·01 to 0·66) | 0·0525 |
| **Secondary case definition (parasitaemia >0 parasites per µL)** | | | | | | |
| Rabies controls (combined) | 112 | 0·26 (39/147·4) | 1 | .. | 1 | .. |
| Delayed RH5.1/Matrix-M | 114 | 0·15 (26/176·5) | 0·44 (0·08 to 0·66) | 0·0226 | 0·44 (0·06 to 0·66) | 0·0283 |
| Monthly RH5.1/Matrix-M | 112 | 0·25 (39/158·9) | 0·08 (–0·44 to 0·41) | 0·7280 | 0·07 (–0·46 to 0·41) | 0·7489 |
| **Secondary case definition (parasitaemia >20 000 parasites per µL)** | | | | | | |
| Rabies controls (combined) | 112 | 0·17 (28/164·3) | 1 | .. | 1 | .. |
| Delayed RH5.1/Matrix-M | 114 | 0·05 (10/194·5) | 0·69 (0·35 to 0·85) | 0·0017 | 0·68 (0·33 to 0·85) | 0·0024 |
| Monthly RH5.1/Matrix-M | 112 | 0·09 (16/183·0) | 0·47 (0·03 to 0·71) | 0·0410 | 0·52 (0·12 to 0·74) | 0·0186 |
| **Additional case definition (parasitaemia >50 000 parasites per µL)** | | | | | | |
| Rabies controls (combined) | 112 | 0·11 (19/178·7) | 1 | .. | 1 | .. |
| Delayed RH5.1/Matrix-M | 114 | 0·02 (4/203·4) | 0·81 (0·44 to 0·94) | 0·0026 | 0·79 (0·38 to 0·93) | 0·0047 |
| Monthly RH5.1/Matrix-M | 112 | 0·01 (2/201·9) | 0·90 (0·58 to 0·98) | 0·0017 | 0·91 (0·62 to 0·98) | 0·0011 |
| **Additional case definition (parasitaemia >100 000 parasites per µL)** | | | | | | |
| Rabies controls (combined) | 112 | 0·06 (11/190·2) | 1 | .. | 1 | .. |
| Delayed RH5.1/Matrix-M | 114 | 0·01 (2/206·3) | 0·83 (0·23 to 0·96) | 0·0213 | 0·82 (0·18 to 0·96) | 0·0271 |
| Monthly RH5.1/Matrix-M | 112 | 0·00 (1/203·1) | 0·91 (0·33 to 0·99) | 0·0193 | 0·92 (0·39 to 0·99) | 0·0148 |

*Event is the first episode of clinical malaria. Event rate per 100 days is (the total number of events/[total child-days of follow-up/100]).

*Table 2:* **Analysis of time to the first episode of clinical malaria from 14 days to 6 months after the third vaccination**

p=0·023) was observed in the delayed group and 8% (–44 to 41%; p=0·73) in the monthly group. Additionally, a parasitaemia of more than 20 000 per µL was examined; here, a vaccine efficacy of 69% (35 to 85%; p=0·0017) was observed in the delayed group and 47% (3 to 71%; p=0·041) in the monthly group (table 2; figure 2B, C). In light of these results, additional post-hoc analyses were performed for case definitions of clinical malaria with a parasitaemia of more than 50 000 per µL; here, a vaccine efficacy of 81% (44 to 94%; p=0·0026) was observed in the delayed group and 90% (58 to 98%; p=0·0017) in the monthly group; and a parasitaemia of more than 100 000 per µL; here, a vaccine efficacy of 83% (23 to 96%; p=0·021) was observed in the delayed group and 91% (33 to 99%; p=0·019) in the monthly group (table 2; figure 2D, E). Secondary analyses of all efficacy endpoints for the intention-to-treat sample showed similar results to those for the per-protocol sample (appendix pp 37–42, 45).

There were no serious adverse events, adverse events of special interest, or suspected unexpected serious adverse reactions reported up to 6 months after the third vaccination. There were no safety concerns raised by the data safety monitoring board after a review of 7 days' data after the vaccination of the first 100 participants, and no further safety reviews were required. Swelling was the most common local solicited adverse event, reported after 24 (3%) of 696 RH5.1/Matrix-M vaccinations, with significantly more swelling reported in the delayed RH5.1/Matrix-M group compared with the combined control groups (OR 11·2; 95% CI 2·6–49·4, p=0·0014). The most common systemic solicited adverse event was fever, reported after 97 (14%) of 696 RH5.1/Matrix-M vaccinations and five (1%) of 349 rabies vaccinations (table 3). There were significantly more fevers in both the delayed (OR 14·1; 95% CI 5·3–37·1, p<0·0001) and monthly (9·7; 3·7–25·9, p<0·0001) RH5.1/Matrix-M vaccination groups compared with the control groups. No participants had febrile convulsions. Most solicited adverse events were mild to moderate in severity. Four participants (2%) of 239 were reported to have severe pain after RH5.1/Matrix-M vaccination, compared with four participants (3%) of 122 after the rabies vaccination. Three participants (1%) of 239 were reported to have severe fever and one participant (<1%) of 239 was reported to have a severe loss of appetite after RH5.1/Matrix-M vaccination (appendix pp 46–47).

For unsolicited adverse events, 39 MedDRA terms were assigned in the 28 days after each of the three vaccinations and there were no significant differences in the number of events per group (appendix pp 48–51). All were classified as unrelated to the vaccines, except for a single episode of moderate fever in the delayed third-dose group within 28 days of vaccination. No biochemical findings of significant concern were found in any group during the vaccination and follow-up period.

During the efficacy follow-up period, prevalent moderate or severe anaemia was assessed at 2 months

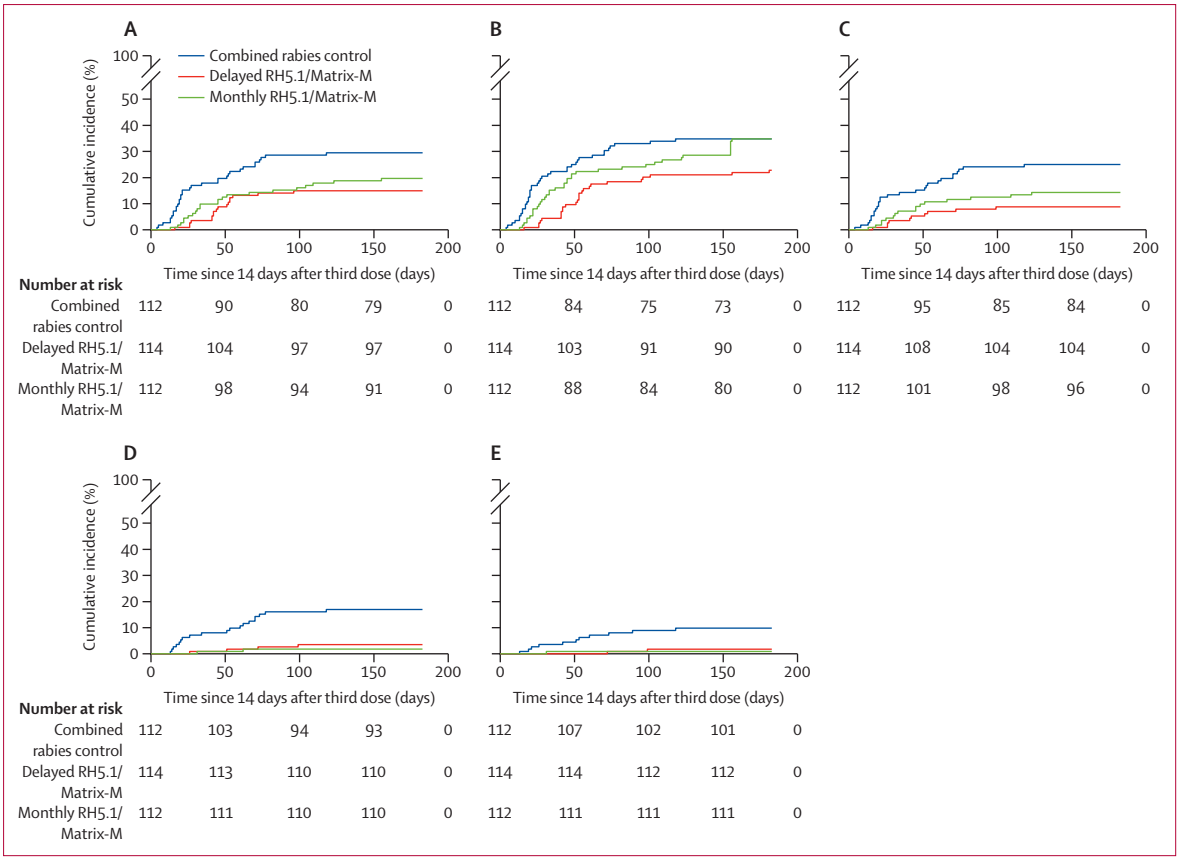


***Figure 2:*** **Kaplan–Meier curves showing the time to the first episode of clinical malaria from 14 days to 6 months after the third vaccination**
The primary analysis was based on the per-protocol sample. (A) Primary case definition of clinical malaria with parasitaemia of more than 5000 parasites per µL. Secondary case definition of clinical malaria with parasitaemia of more than 0 parasites per µL (B), or more than 20 000 parasites per µL (C). Additional case definition of clinical malaria with parasitaemia of more than 50 000 parasites per µL (D), or more than 100 000 parasites per µL (E).

and 6 months after the third vaccination. There were no cases of severe anaemia (haemoglobin <5·0 g/dL), no participants requiring blood transfusion, and no significant differences in the frequency of moderate anaemia (haemoglobin <8·0 g/dL) between the groups (appendix p 52). There were no cases of severe malaria in any participant in the 6 months after the third vaccination.

At baseline, 356 (99%) of 360 participants had background-level anti-RH5.1 serum IgG antibody responses of less than 0·2 µg/mL; the other four participants had responses of less than 1 µg/mL. At 14 days after the third vaccination, responses were similar to baseline in the combined rabies vaccine control groups. In contrast, high responses were seen in the delayed regimen RH5.1/Matrix-M group (geometric mean anti-RH5.1 IgG concentration 837 µg/mL; IQR 326–2200); these were significantly higher compared with the monthly regimen group (geometric mean 626 µg/mL; 222–1455; p=0·0006; figure 3A). In vitro functional anti-parasitic activity was assessed by the GIA assay at a total purified IgG concentration of 2·5 mg/mL at 14 days after the third vaccination. Samples from control participants showed negligible GIA (<20%), apart from three participants who had GIA of more than 50%. Mean GIA in the delayed regimen RH5.1/Matrix-M group was 79·0% (SD 14·3), significantly higher than the mean GIA in the monthly group of 74·2% (15·9; p=0·016; figure 3B, appendix pp 53–54).

## Discussion

Here, we report that the standalone blood-stage vaccine candidate RH5.1/Matrix-M, delivered in a delayed third-dose (0, 1, and 5 month) regimen, shows significant efficacy of 55% (95% CI 20–75%) against clinical malaria in the target population of African children over a 6-month follow-up. The vaccine was also well tolerated and no serious adverse events were reported up to 6 months after the third vaccination. Data have been reported for a total of 109 adults, 305 children, and 18 infants vaccinated with RH5-based vaccines in the UK, Tanzania, and Burkina Faso,[14,18–20] which all show similar safety and tolerability profiles, whereas Matrix-M adjuvant is now licensed in vaccines for malaria and COVID-19.[3,23] Ongoing phase 1 and 2 trials continue to monitor the safety of RH5.1/Matrix-M vaccination.

| | Delayed rabies control | Delayed RH5.1/ Matrix-M | Monthly rabies control | Monthly RH5.1/ Matrix-M |
|---|---|---|---|---|
| **Number of participants** | | | | |
| Dose 1 | 62 | 119 | 60 | 120 |
| Dose 2 | 61 | 118 | 60 | 119 |
| Dose 3 | 55 | 114 | 57 | 113 |
| **Local events** | | | | |
| Pain | | | | |
| Dose 1 | 3 (5%) | 5 (4%) | 1 (2%) | 0 |
| Dose 2 | 1 (2%) | 6 (5%) | 0 | 1 (1%) |
| Dose 3 | 0 | 3 (3%) | 1 (2%) | 3 (3%) |
| Swelling | | | | |
| Dose 1 | 1 (2%) | 0 | 0 | 0 |
| Dose 2 | 0 | 17 (14%) | 1 (2%) | 5 (4%) |
| Dose 3 | 0 | 2 (2%) | 0 | 0 |
| Redness | | | | |
| Dose 1 | 0 | 0 | 1 (2%) | 0 |
| Dose 2 | 0 | 1 (1%) | 0 | 2 (2%) |
| Dose 3 | 0 | 0 | 0 | 0 |
| Warmth | | | | |
| Dose 1 | 2 (3%) | 3 (3%) | 0 | 0 |
| Dose 2 | 0 | 2 (2%) | 0 | 4 (3%) |
| Dose 3 | 1 (2%) | 1 (1%) | 0 | 1 (1%) |
| **Systemic events** | | | | |
| Fever | | | | |
| Dose 1 | 1 (2%) | 6 (5%) | 0 | 4 (3%) |
| Dose 2 | 2 (3%) | 27 (23%) | 1 (2%) | 26 (22%) |
| Dose 3 | 1 (2%) | 21 (18%) | 0 | 13 (12%) |
| Irritable | | | | |
| Dose 1 | 0 | 0 | 0 | 0 |
| Dose 2 | 1 (2%) | 2 (2%) | 1 (2%) | 0 |
| Dose 3 | 1 (2%) | 1 (1%) | 1 (2%) | 1 (1%) |
| Drowsiness | | | | |
| Dose 1 | 0 | 4 (3%) | 1 (2%) | 0 |
| Dose 2 | 0 | 3 (3%) | 0 | 1 (1%) |
| Dose 3 | 0 | 0 | 0 | 1 (1%) |
| Loss of appetite | | | | |
| Dose 1 | 1 (2%) | 2 (2%) | 0 | 0 |
| Dose 2 | 2 (3%) | 4 (3%) | 1 (2%) | 1 (1%) |
| Dose 3 | 1 (2%) | 1 (1%) | 0 | 1 (1%) |

Data are n or n (%).

***Table 3:*** **Solicited adverse events within 7 days of vaccine dose across all groups**

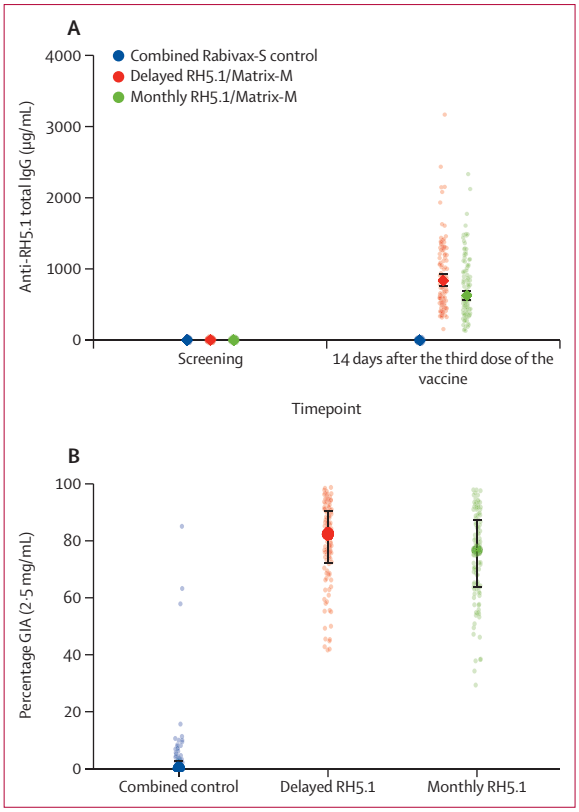


***Figure 3:*** **RH5.1/Matrix-M humoral immunogenicity**
Immunological outcomes in study participants in the per-protocol sample. (A) Anti-RH5.1 serum IgG responses by vaccination group at baseline (screening) and day 14 after the third vaccine dose. Individual anti-RH5.1 total IgG antibody concentrations (dots) and geometric mean with 95% CIs (diamonds with black bars). N=111 children for the rabies (Rabivax-S) delayed and monthly groups combined, N=113 for the delayed RH5.1/Matrix-M group, and N=107 for the monthly RH5.1/Matrix-M group. (B) Percentage in vitro GIA of 3D7 clone *Plasmodium falciparum* parasites by vaccination group, using 2·5 mg/mL total IgG purified from serum taken on day 14 after the third vaccine dose. Individual percentage inhibition figures (small dots) and median and IQR (large dots and black bars). N=97 children for the rabies (Rabivax-S) delayed and monthly groups combined, N=113 children for the delayed RH5.1/Matrix-M group, and N=108 for the monthly RH5.1/Matrix-M group. GIA=growth inhibition activity.

Here we studied RH5.1/Matrix-M efficacy for the first time in an area of seasonal malaria transmission, with vaccinations completed part way through the malaria season. Previously published phase 2 field efficacy trials of *P falciparum* blood-stage vaccine candidates, targeting various antigens including AMA1, $MSP1_{42}$, MSP2, GLURP, MSP3, and RESA, all yielded disappointing results, reporting either no or minimal clinical efficacy, or in some cases evidence of strain-specific efficacy linked to target antigen polymorphism.[5–9] Other, more recent, clinical blood-stage vaccine candidates targeting antigens such as full-length MSP1[24] or SERA5[25] have not been assessed yet in phase 2 trials. Given these phase 2 studies were done over the last 25 years in different settings with different transmission patterns and in children of different age ranges, it is not possible to directly compare with our study. However, our results show that a standalone blood-stage vaccine can achieve significant efficacy against clinical malaria in children aged 5–17 months, in line with the approved age range for use of the pre-erythrocytic vaccines RTS,S/AS01 and R21/Matrix-M, and now enabling the future assessment of combination malaria vaccine strategies targeting two stages of the parasite's lifecycle. A second-generation multi-stage paediatric vaccination strategy offers hope for higher

and more durable efficacy against clinical malaria, especially if the pre-erythrocytic and blood-stage components act additively, or even synergistically.

Seasonal malaria chemoprevention was given to children in the study area as part of a programme by local health services, as per national policy recommendation, but coverage was suboptimal. Our study documented seasonal malaria chemoprevention uptake, but did not deliver any further seasonal malaria chemoprevention. It will be important in the future to test RH5.1/Matrix-M efficacy in settings where there is no seasonal malaria chemoprevention, and to also establish in seasonal settings whether the combination of blood-stage and pre-erythrocytic vaccines can result in a better outcome (than a pre-erythrocytic vaccine alone) as indicated by studies of RTS,S/AS01 and seasonal malaria chemoprevention.[26]

Our study was not powered to show a difference in vaccine efficacy between the 0, 1, and 2 and 0, 1, and 5 month delivery regimens with RH5.1/Matrix-M. However, the vaccine efficacy for the 0, 1, and 5 month regimen was higher than for the 0, 1, and 2 month regimen when using the primary case definition for clinical malaria, albeit with overlapping 95% CIs, and this is consistent with the 0, 1, and 5 month regimen inducing higher IgG concentrations and GIA. The difference in vaccine efficacy was larger at 6 months than 3 months, suggesting the 0, 1, and 5 month regimen might offer more durable protection. Previous trials of RTS,S/AS01 reported improved efficacy against malaria challenge in healthy US adults when using a 0, 1, and 7 month regimen with antigen and adjuvant fractionated for the delayed third-dose regimen (as opposed to 0, 1, and 2 month dosing);[27] however, this did not translate to improved field efficacy in children aged 5–17 months.[28] In line with these observations, our previous phase 1b trial data with RH5.1/Matrix-M in Tanzanian children suggested that a delayed (full) third dose, as opposed to monthly dosing or a delayed fractional third dose, might induce more robust and durable antibody responses.[20] Non-human primate data with adjuvanted R21 vaccine also suggest that delayed boosting can improve serum antibody durability.[29] Ongoing analyses in this trial will thus continue to investigate whether the delayed third-dose regimen induces more durable immunity in contrast with monthly dosing.

Notably, our secondary and post-hoc analyses showed a lower vaccine efficacy (compared with the primary endpoint) when we used the secondary clinical malaria case definition of any parasitaemia (>0 parasites per µL) but increasingly improved efficacy when we used secondary or additional malaria case definitions with a higher parasitaemia cutoff of more than 20 000 parasites per µL, more than 50 000 parasites per µL, or more than 100 000 parasites per µL. The delayed third-dose RH5.1/Matrix-M regimen showed significant efficacy against all definitions, whereas the monthly regimen only reached significance at the higher cutoffs, consistent with the more modest performance of this regimen, but nonetheless suggesting a biological effect. These data appear in line with research in animal vaccination and challenge models of malaria,[13] whereby blood-stage malaria vaccines can reduce peak parasitaemia. This finding would also not be expected to occur with pre-erythrocytic vaccines; post-hoc analysis of data from the phase 3 trial of R21/Matrix-M[3] showed almost identical vaccine efficacy for all the cutoff levels of parasitaemia analysed here (Adrian Hill, University of Oxford, UK, personal communication). Consequently, our data show that RH5.1/Matrix-M can partly protect against clinical malaria but can also reduce blood-stage parasitaemia in clinical cases. This finding might have implications for the prevention of severe or life-threatening disease in the real-world setting when pre-erythrocytic or blood-stage vaccine-induced immunity to clinical malaria fails or wanes.

Solicited adverse event rates observed with RH5.1/Matrix-M in this phase 2b trial compare favourably with data reported in the much larger phase 3 trials of R21/Matrix-M and RTS,S/AS01.[2,3] Swelling at the injection site, the most common local solicited adverse event in this trial, occurred after 4% of vaccinations with R21/Matrix-M and 10% with RTS,S/AS01, in comparison with 3% with RH5.1/Matrix-M. For R21/Matrix-M and RTS,S/AS01, the most common local solicited adverse event was pain, occurring after 19% and 12% of vaccinations, respectively, in comparison with 3% with RH5.1/Matrix-M. Fever was the most common systemic solicited adverse event with all vaccines, occurring after 47% of vaccinations with R21/Matrix-M and 31% with RTS,S/AS01, in comparison with 14% with RH5.1/Matrix-M.

The absence of RH5 serum antibody responses at baseline or in the control groups at 14 days after the third vaccination is consistent with the known sero-epidemiology and sequence conservation of RH5, suggesting this antigen is not a dominant target of naturally acquired malaria immunity.[10,18,30] In contrast, the RH5.1/Matrix-M vaccine candidate was highly immunogenic for functional anti-RH5.1 serum IgG antibody across both dosing regimens, with the delayed third-dose regimen showing small but significant improvements in the ELISA and GIA responses (compared with the monthly regimen) 14 days after the third vaccination, in line with our data seen in Tanzanian children in the RH5.1/Matrix-M phase 1b study.[20]

The mean GIA observed in both RH5.1/Matrix-M groups, and more than 80% of individual children, at this timepoint also exceeded 60% GIA measured at 2·5 mg/mL total IgG (appendix pp 53–54), a threshold level we previously reported as required for protection after RH5 vaccination and *P falciparum* blood-stage challenge in *Aotus* monkeys.[13,15] These data are thus consistent with both RH5.1/Matrix-M regimens showing significant efficacy against clinical malaria in the first

3 months after the third vaccination. Analysis of the kinetic of both groups' immune responses beyond this peak post-vaccination timepoint are ongoing, but the efficacy data after 3 months suggest that differences might be seen with respect to serum antibody durability or the possibility for natural boosting of the vaccine-induced response.

This study has limitations, including the absence of quantitative PCR data at this stage; however, secondary endpoints relating to efficacy against asymptomatic infection will be reported at the end of the trial. With the completion of vaccine doses part way through the malaria season, it is also possible that vaccine efficacy might be different if administered earlier—namely, with the primary vaccination series (all three doses) being completed before the season. Another limitation that comes from administering the vaccine part way through the malaria season is that there was insufficient follow-up time to observe many children having multiple episodes. It is possible that naturally acquired immunity might interact with vaccine-induced immunity, protecting children from subsequent episodes, but the current analysis was unable to investigate this. Nonetheless, follow-up of the VAC091 trial is continuing to establish the efficacy at 12 months after the third vaccination, and to assess the durability of the vaccine-induced immune response and the potential effect of natural malaria exposure. We will also administer a fourth (booster) dose of vaccine at 12 months to groups 1–4 to enable efficacy monitoring over a second year of follow-up. We also limited the age range of participants in this trial to 5–17 months, to align with earlier studies of RTS,S/AS01 and R21/Matrix-M. A wider age range, inclusive of younger infants and older children, will be covered in future trials. We have not yet assessed RH5.1/Matrix-M delivered in an age-based (non-seasonal) administration schedule, or in sites with lower or higher levels of perennial malaria transmission compared with the seasonal setting in Nanoro; this will be addressed in future studies. We have also not yet formally analysed our immunological datasets for correlates of protection; this, along with an assessment for any evidence of *P falciparum* RH5 sequence selection in participants who have been vaccinated versus controls, is the focus of ongoing work.

#### Contributors

JS, SES, SJD, and AMM conceived the trial. HT was the trial principal investigator. AMM was the chief investigator, and SJD was the senior laboratory investigator. HMN, JS, AS, SD, OT, TR, FO, EO, KCSD, NAK, ZMJC, MK, MdAB, FK, HS, CMN, DP, AD, CAL, KM, SES, HT, and AMM contributed to the implementation of the study and data collection. SS and JB analysed data and completed the statistical analysis. HMN, SD, J-SC, AML, KS, and RR managed the project. LDWK, US, and KS assisted with vaccine provision. HMN, JS, KM, SES, SJD, HT, and AMM interpreted the data, contributed to writing the manuscript, and accessed and verified the data. 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

LDWK and SJD are named inventors on patent applications relating to RH5 malaria vaccines. AMM has an immediate family member who is an inventor on patent applications relating to RH5 malaria vaccines. US is an employee of Serum Institute of India, the manufacturer of the R21/Matrix-M vaccine. All other authors declare no competing interests.

#### Data sharing

Data associated with this study are present in the paper or appendix and will be available after the end of the study upon reasonable request that should be directed to the corresponding author (angela.minassian@bioch.ox.ac.uk). Proposals will be reviewed and approved by the sponsor, chief investigator, and collaborators. After the approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. Any shared data will be de-identified.

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#### References

1 WHO. World malaria report 2023. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023 (accessed Nov 25, 2024).
2 RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 2015; **386:** 31–45.
3 Datoo MS, Dicko A, Tinto H, et al. Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, phase 3 trial. *Lancet* 2024; **403:** 533–44.
4 Draper SJ, Sack BK, King CR, et al. Malaria vaccines: recent advances and new horizons. *Cell Host Microbe* 2018; **24:** 43–56.
5 Sirima SB, Mordmüller B, Milligan P, et al. A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children. *Vaccine* 2016; **34:** 4536–42.
6 Thera MA, Doumbo OK, Coulibaly D, et al. A field trial to assess a blood-stage malaria vaccine. *N Engl J Med* 2011; **365:** 1004–13.
7 Sagara I, Dicko A, Ellis RD, et al. A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. *Vaccine* 2009; **27:** 3090–98.
8 Ogutu BR, Apollo OJ, McKinney D, et al. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in western Kenya. *PLoS One* 2009; **4:** e4708.
9 Genton B, Betuela I, Felger I, et al. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. *J Infect Dis* 2002; **185:** 820–27.
10 Douglas AD, Williams AR, Illingworth JJ, et al. The blood-stage malaria antigen PfRH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat Commun* 2011; **2:** 601.

11 Crosnier C, Bustamante LY, Bartholdson SJ, et al. Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 2011; **480:** 534–37.

12 Galaway F, Yu R, Constantinou A, Prugnolle F, Wright GJ. Resurrection of the ancestral RH5 invasion ligand provides a molecular explanation for the origin of *P. falciparum* malaria in humans. *PLoS Biol* 2019; **17:** e3000490.

13 Douglas AD, Baldeviano GC, Lucas CM, et al. A PfRH5-based vaccine is efficacious against heterologous strain blood-stage *Plasmodium falciparum* infection in aotus monkeys. *Cell Host Microbe* 2015; **17:** 130–39.

14 Minassian AM, Silk SE, Barrett JR, et al. Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. *Med (NY)* 2021; **2:** 701–19.e19.

15 Douglas AD, Baldeviano GC, Jin J, et al. A defined mechanistic correlate of protection against *Plasmodium falciparum* malaria in non-human primates. *Nat Commun* 2019; **10:** 1953.

16 Thiam LG, McHugh K, Ba A, et al. Vaccine-induced human monoclonal antibodies to PfRH5 show broadly neutralizing activity against *P. falciparum* clinical isolates. *NPJ Vaccines* 2024; **9:** 198.

17 Barrett JR, Pipini D, Wright ND, et al. Analysis of the diverse antigenic landscape of the malaria protein RH5 identifies a potent vaccine-induced human public antibody clonotype. *Cell* 2024; **187:** 4964–80.e21.

18 Payne RO, Silk SE, Elias SC, et al. Human vaccination against RH5 induces neutralizing antimalarial antibodies that inhibit RH5 invasion complex interactions. *JCI Insight* 2017; **2:** 96381.

19 Silk SE, Kalinga WF, Mtaka IM, et al. Superior antibody immunogenicity of a viral-vectored RH5 blood-stage malaria vaccine in Tanzanian infants as compared to adults. *Med (NY)* 2023; **4:** 668–86.e7.

20 Silk SE, Kalinga WF, Salkeld J, et al. Blood-stage malaria vaccine candidate RH5.1/Matrix-M in healthy Tanzanian adults and children; an open-label, non-randomised, first-in-human, single-centre, phase 1b trial. *Lancet Infect Dis* 2024; **24:** 1105–17.

21 Jin J, Tarrant RD, Bolam EJ, et al. Production, quality control, stability, and potency of cGMP-produced *Plasmodium falciparum* RH5.1 protein vaccine expressed in *Drosophila* S2 cells. *NPJ Vaccines* 2018; **3:** 32.

22 Malkin EM, Diemert DJ, McArthur JH, et al. Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for *Plasmodium falciparum* malaria. *Infect Immun* 2005; **73:** 3677–85.

23 Heath PT, Galiza EP, Baxter DN, et al. Safety and efficacy of NVX-CoV2373 COVID-19 vaccine. *N Engl J Med* 2021; **385:** 1172–83.

24 Blank A, Fürle K, Jäschke A, et al. Immunization with full-length *Plasmodium falciparum* merozoite surface protein 1 is safe and elicits functional cytophilic antibodies in a randomized first-in-human trial. *NPJ Vaccines* 2020; **5:** 10.

25 Ouédraogo A, Bougouma EC, Palacpac NMQ, et al. Safety and immunogenicity of BK-SE36/CpG malaria vaccine in healthy Burkinabe adults and children: a phase 1b randomised, controlled, double-blinded, age de-escalation trial. *Front Immunol* 2023; **14:** 1267372.

26 Chandramohan D, Zongo I, Sagara I, et al. Seasonal malaria vaccination with or without seasonal malaria chemoprevention. *N Engl J Med* 2021; **385:** 1005–17.

27 Regules JA, Cicatelli SB, Bennett JW, et al. Fractional third and fourth dose of RTS,S/AS01 malaria candidate vaccine: a phase 2a controlled human malaria parasite infection and immunogenicity study. *J Infect Dis* 2016; **214:** 762–71.

28 Samuels AM, Ansong D, Kariuki SK, et al. Efficacy of RTS,S/AS01$_E$ malaria vaccine administered according to different full, fractional, and delayed third or early fourth dose regimens in children aged 5–17 months in Ghana and Kenya: an open-label, phase 2b, randomised controlled trial. *Lancet Infect Dis* 2022; **22:** 1329–42.

29 Arunachalam PS, Ha N, Dennison SM, et al. A comparative immunological assessment of multiple clinical-stage adjuvants for the R21 malaria vaccine in nonhuman primates. *Sci Transl Med* 2024; **16:** eadn6605.

30 Osier FH, Mackinnon MJ, Crosnier C, et al. New antigens for a multicomponent blood-stage malaria vaccine. *Sci Transl Med* 2014; **6:** 247ra102.