

Immune responses elicited by R21/Matrix-M™ malaria vaccine in children aged 5-36 months: robust one year durability and correlation between long-lived CSP-IgG and vaccine efficacy

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Immune responses elicited by R21/Matrix-M™ malaria vaccine in children aged 5-36 months: robust one year durability and correlation between long-lived CSP-IgG and vaccine efficacy.

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Abstract

Malaria vaccines are a critical addition to global efforts to reduce disease burden, yet our understanding of the durability and immunologic mechanism of action across diverse endemic settings is limited. Here we evaluate humoral immune responses to four components of the R21/Matrix-M™ vaccine following a three-dose primary series and one booster dose administered a year later to children aged 5-36 months across five sites in four malaria-endemic African countries.

Here we report robust induction of vaccine-specific antibodies after the primary series and booster dose of R21/Matrix-M™. Younger children aged 5-17 months had higher fold change from pre-vaccination to post-primary series for circumsporozoite protein (CSP)-specific IgG compared with 18-36 month olds ($p < 0.001$) but there was no effect of age on the waning of antibodies in the first year. While baseline parasitemia was associated with higher pre-vaccination anti-CSP IgG titres ($p < 0.001$), pre-vaccination CSP-specific antibodies did not impair the ability of the vaccine to elicit robust antibody responses.

. Both C-terminal CSP-specific and NANP-repeat IgG titres at 28 and 180 days after the primary series were associated with a reduction in the rate of clinical malaria in the R21/Matrix-M™ study arm. Notably, at one year following primary series, C-terminal CSP-specific IgG titres continued to be associated with a reduction in the rate of clinical malaria (IRR 0.32 (95% CI: 0.17, 0.61), underscoring its potential as a biomarker of vaccine-induced protection.

Introduction

Malaria is caused by parasites of the genus *Plasmodium* and is transmitted by *Anopheles* mosquitoes. In 2024 malaria was responsible for an estimated 282 million clinical cases (an 8.6% increase on 2023) and 610,000 deaths (an 11.9% increase compared to 2023) despite widespread provision of insecticide-treated bed nets and increased access to anti-malarial drugs (1,2). The World Health Organisation (WHO) African Region accounted for 94% of global malaria cases and 95% of global malaria deaths in 2023 with 73.7% of these malaria deaths among children aged under 5 years of age (1,2). 2020 goals set by the Global Technical Strategy for Malaria 2016-2030 were not achieved and updated targets were set in 2021 to include a 90% reduction of malaria mortality and case incidence globally by 2030 compared with 2015 (3). Vaccines will be a key additional tool to reduce the burden of disease, and to combat the rise of antimalarial drug resistance (4,5).

Two malaria vaccines; Mosquirix (RTS,S/AS01, GSK), and R21/Matrix-M™ (Oxford University and Serum Institute of India), have been pre-qualified by the WHO and commenced roll-out in January and May 2024, respectively. Both vaccines address the first stage of the parasite life cycle by targeting the circumsporozoite protein (CSP) of *Plasmodium falciparum* (*P.f*), the species of malaria primarily responsible for mortality in Africa (1). As of March 2026 the price of R21/Matrix-M is less than half that of RTS,S (\$3.9 vs \$9.3 per dose)¹. Vaccines targeting the blood stage and mosquito stage are also in development with promising potential for inclusion into a multi-stage combination formulation. (6,7)

Using a three-dose vaccine regimen given at one-month intervals, R21/Matrix-M™ demonstrated 12-month vaccine efficacy against clinical malaria of 79% (95% CI, 73-84%) in seasonal malaria transmission sites, and 75% (95% CI, 65-83%) in perennial malaria transmission sites among children aged 5-17 months. In children aged 18-36 months over the same period, vaccine efficacy was 73% (95% CI, 67-78%) in seasonal transmission sites, and 63% (95% CI, 53-72%) in perennial malaria transmission sites (8). Individual site vaccine efficacy estimates for children aged 5-36 months in standard sites were 54% in Bagamoyo, Tanzania, 58% in Kilifi, Kenya, and 73% in Dandé, Burkina Faso. For seasonal sites, vaccine efficacy was 72% in Nanoro, Burkina Faso and 82% in Bougouni, Mali.(8)

The CSP antigen comprises three main domains: an N-terminal domain, a central repeat region composed of between 25-40 repeating Asn-Ala-Asn-Pro (NANP) tetrapeptides interspersed with an NPDP tetrapeptide and two to four NVDP tetrapeptides, and a C-terminal domain containing a conserved α -thrombospondin type-1 repeat domain (9). For both RTS,S/AS01 and R21/Matrix-M™ vaccines, elevated IgG antibodies against CSP epitopes are associated with reduced clinical risk (8,10–13). However, in a controlled human malaria infection study carried out in malaria-naïve adult participants in the UK, R21/Matrix-M™-induced IgG specific to the NANP repeat sequence did not constitute an individual-level correlate of protection with a defined threshold (14).

Here we explore the immunogenicity results from the R21/Matrix-M™ Phase 3 clinical trial in greater detail. We describe the temporal dynamics of IgG antibody titres specific to two CSP

¹ <https://www.unicef.org/supply/documents/malaria-vaccine-price-data>

regions (NANP and C-Term), Hepatitis B surface antigen (HBsAg), and the full-length R21 vaccine construct, measured at five time points from pre-vaccination to 28 days post booster dose. We report how these antibody responses vary among different demographic subgroups and evaluate their relationship with malaria incidence. Additionally, we examine the correlations between antibody titres targeting different vaccine domains to better understand the immunological profile elicited by the vaccine. This comprehensive analysis provides insights into the immunogenicity of the R21/Matrix-M™ vaccine and its effectiveness across diverse target populations.

Methods

Trial design

The samples included in this analysis were collected from an ongoing Phase 3 clinical trial which commenced in 2021 (ClinicalTrials.gov, NCT04704830). The trial methodology is described in full elsewhere (8). In brief, 4878 children aged 5-36 months were enrolled from five sites, two with seasonal malaria transmission (Nanoro and Bougouni) and three with perennial malaria transmission (Dandé, Bagamoyo, and Kilifi – with the latter two sites approximately half the size of the other three). Children were randomly assigned at a ratio of 2:1 to receive a primary series of three doses of either 5µg R21 plus 50 µg Matrix-M or control vaccine (the rabies vaccine Abhayrab), given at one-month intervals. A booster dose of the same vaccine was administered 12 months after the third dose of the primary series. In seasonal sites the primary series, and subsequent boosters, were administered shortly before the start of the malaria transmission season ; whereas an age-based vaccination schedule was used in the perennial transmission sites. Seasonal malaria chemoprevention (SMC) was administered according to local guidelines to children in both randomisation arms outside of the clinical study protocol.

The first 50% of participants recruited in each site had blood samples taken for immunological analyses and formed the cohort analysed in this paper (Figure 1). Samples were taken pre-vaccination (PV), 28 days post primary series (D28, -7/+14 days), 180 days post primary series (D180, -21/+28 days), day of booster dose prior to receiving the booster (B0, booster was given 365 days post primary series (±35 days)) and 28 days post booster dose (B28, ±7 days) (Supplementary Figure S1).

The data presented here are from the modified per-protocol (MPP) population (8) defined as participants who received the second vaccine dose 3-6 weeks after the first, and who received their third dose 3-16 weeks after the second. The longer interval between the second and third dose was due to a temporary pause at the Dandé site while national regulatory authority clarifications took place. In the immunogenicity cohort, 16% of Dandé participants received their third dose >6 weeks after their second dose. Individuals are included in the MPP population post-booster if they received the booster dose 365 ±35 days after the third dose.

Sample selection

All available PV and D28 samples collected within the visit window were analysed for immunogenicity. Thereafter, a minimum of 30% of samples per site from the control arm and 50%

of samples per site from the R21/Matrix-M™ arm were randomly selected for analysis from the later time points: D180, B0 and B28 (Supplementary Table S1). Due to availability of assay reagents, it was not possible to analyse the full 100% of samples at later timepoints. Any samples collected outside the visit window specified in the protocol were not included in analysis. (8)

Immunogenicity assessments

Serum (Bougouni and Dandé) and plasma (Nanoro, Bagamoyo, and Kilifi) were separated from whole blood by centrifugation and antibody responses to vaccine antigens were measured using a Meso-Scale Discovery (MSD) immune assay. This multiplex assay measures serum or plasma IgG antibody binding to four antigens; six repeats of the NANP repeat region of *P. f* CSP; the C-terminus (C-term) region of CSP; full length R21 vaccine construct; and hepatitis B surface antigen (HBsAg) as described previously (15). NANP, C-term and full-length R21 are referred to as CSP antigens. Briefly, plates were blocked with 150µL/well PBS-casein for 30 minutes at room temperature (shaking at 400 rpm) and washed three times with 0.05% PBS-Tween. Pre-diluted test samples, quality control samples, and standard curve were added to the plates at 50µL/well and incubated with shaking for two hours at room temperature. Plates were washed three times with 0.05% PBS-Tween, and 50µL/well of SULFO-TAG™ Anti-human IgG (1µg/mL; MSD #R32AJ-1) was added. Plates were incubated with shaking for one hour at room temperature and washed three times with PBS-Tween 0.5%, and 150µL/well MSD GOLD Read Buffer (MSD #R60AM-4) was added before plates were immediately read using a QuickPlex SQ 120MM with Methodical Mind software.

One biological sample per participant per timepoint was assayed in triplicate at three dilutions by laboratory staff blinded to vaccine group allocation. Plates were deemed to have passed if the seven-point standard curve and three quality control samples all passed in house criteria according to assay validation (15). Samples were deemed to have passed if triplicate measurements reported equal or less than 20%CV and if the value lay within the linear portion of the standard curve.

Statistical analysis

Geometric mean titres (GMT) and 95% confidence intervals (CIs) were used to summarise IgG antibody titres for four antigens (NANP, C-term, full length R21, and HBsAg) at five timepoints (PV, D28, D180, B0 & B28) stratified by study arm, site, and age group (5-17 vs 18-36 months at randomisation). Differences in geometric mean IgG antibody titres between age groups were assessed using a linear regression model of log10 transformed IgG antibody titres, adjusting for site and including an interaction term between study arm and age group. The percentage boost of antigen-specific IgG antibodies were calculated by dividing the titre at B28 by the titre at D28, and multiplying by 100. The median of these individual level percentage boosts is presented.

Geometric mean fold change in IgG antibody titres from PV to D28 and 95% confidence interval were calculated for all four antigens, stratified by site, gender, age group, nutritional status (underweight (defined as having a weight-for-age z-score at screening of <-2) vs normal weight) and number of malaria cases in the first year (none vs at least one). Clinical malaria cases were defined as an axillary temperature $\geq 37.5^{\circ}\text{C}$ and/or history of fever within the last 24 hours, and

microscopically confirmed *P.f* asexual parasite density >5000 parasites/μl. The difference in fold change between the sub-groups listed above was assessed using a linear regression model of the log10 transformed fold rise adjusting for site, gender, age group, nutritional status and number of malaria cases (none vs at least one).

To determine the percentage reduction in antigen-specific IgG titre over time within the R21/Matrix-M™ study arm, a linear regression with unstructured correlated residuals was used to account for repeated measures within individuals. The model included fixed effects for timepoint (PV, D28, D180, B0 & B28). The average percentage reduction per week, and 95% CI, between D28 and D180 and between D180 and B0 was reported. To assess the impact of site, gender, age group, nutritional status (underweight vs normal weight) and number of malaria cases in the first year (none vs at least one), an interaction term was included in the above model between the variable of interest and timepoint. A likelihood ratio test comparing the model restricted to data from timepoints D28, D180 and B0, with and without the interaction term, was used to test if the rate of change in geometric mean IgG titre over time differed between the subgroups. All models were adjusted for site, gender, age group, nutritional status and number of SMC rounds given in 2021.

A linear regression model adjusted for study site was used to investigate the association between log10 anti-NANP, C-term, full length R21 and HBsAg IgG titre at PV and D28 with age in months at randomisation in the R21/Matrix-M™ study arm.

Incidence Rate Ratios (IRR) and 95% CI for the association between log10 antigen-specific IgG at D28, D180 and B0, and number of malaria cases in the first year were calculated using a negative binomial model adjusted for study site and restricted to individuals in the R21/Matrix-M™ study arm. This analysis was conducted separately for IgG antibodies targeted against each of the four antigens. Baseline parasitemia was determined using the result of a malaria blood film from the day of, or up to four days prior to first vaccination. Quantile regression models, adjusted for age, sex, and trial site, were used to test for associations between baseline parasitemia and CSP (NANP, C-Term and full length R21)-specific IgG at PV and D28.

As per Dattoo *et al* (2022)(13), cumulative distribution curves were used to define a threshold correlate of VE per site. The threshold correlate is defined as the level of NANP- or C-Term-specific IgG at D28 on the cumulative distribution curve at 1 minus the level of 12-month Vaccine Efficacy reported for that site. Vaccine efficacy was calculated as 1-hazard ratio from a Cox regression model including all trial participants in the MPP population, adjusting for age at randomisation, sex, bed net use and number of SMC rounds.

Analyses were performed using Stata version 18 (StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC) and R v. 4.3.3 R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. Figures were generated using R v. 4.3.3 and GraphPad Prism (version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com).

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Ethics and consent

Lists of eligible children were identified from local surveillance databases and community sensitisation. Prior to eligibility screening, parents or guardians of participants provided written or thumb-printed consent, which was verbally re-checked at every study visit. Inclusion criteria detailed that participants should be 5-36 months of age at the time of their first vaccination, signed and witnessed informed consent should be obtained from the parent/guardian and that they should plan to remain in the study area for the duration of the trial. Exclusion criteria included significant co-morbidities and receipt of another malaria vaccine. Full details of the eligibility criteria can be found in Dattoo *et al* (8). There was a minimum two-week interval between administration of the study vaccine and any Expanded Programme on Immunisation (EPI) vaccine. The trial protocol was approved by all the local ethics committees and regulatory authorities as well as the ethics committees at the University of Oxford and The London School of Hygiene & Tropical Medicine, with supportive coordination by the African Vaccine Regulatory Forum (AVAREF).

Results

4,644 children aged 5-36 months were included in the MPP analysis population of the R21/Matrix-M™ vaccine Phase 3 trial. Of these, 2,308 (49.7%) were included in the immunogenicity cohort (Figure 1). 2,163 (93.9%) children in the immunogenicity cohort received a booster dose of either R21/Matrix-M™ or control vaccine within 365 ±35 days of the third dose and were therefore included in the MPP population for post-boost analyses.

The demographic characteristics were comparable between the total Phase 3 MPP population and the immunogenicity cohort. Children had a mean age at randomisation of 19.0 months (SD = 9.0) with 48.1% female in the total population, compared with 19.2 months (SD = 9.0) and 48.5% female in the immunogenicity cohort (Table 1). SMC was administered as part of routine care in the West African sites only (Nanoro, Bougouni and Dandé), and administration of the full 4 doses varied between the sites (Supplementary Table S8).

In the R21/Matrix-M™ vaccination arm, valid NANP specific IgG antibody titres were obtained in 97% (1497/1546) of pre-vaccination screening samples, 89% (1369/1546) at D28, 57% (884/1546) at D180, 57% (827/1446) at B0 and 54% (775/1446) at B28 (Supplementary Table S1a; numbers analysed for other antigens can be seen in Supplementary Table S1b-d).

R21/Matrix-M™ vaccination induces robust IgG responses against four vaccine-antigens with heterogeneity among sites

R21/Matrix-M™ vaccinated children from all sites showed increases in geometric mean titre (GMT) for all antigens between PV and D28 timepoints (Supplementary Tables S2a-d). A geometric mean fold change increase of 37295 (34260-40600) was observed for NANP-specific IgG titres at D28 compared to PV (geometric mean fold change for all sites, all age groups combined; Supplementary Table S3a). Individuals in the control vaccine arm did not show a meaningful

increase in IgG titres to any antigen over the follow up period reported here (Figure 2 and Supplementary Tables S2a-d).

IgG titres induced by the primary series of three vaccinations (D28 timepoint) varied between sites. The highest NANP-specific IgG fold change between PV and D28 was seen among children in Bagamoyo (80495, 95% CI: 66164, 97932) and the lowest in Dandé (21193, 95% CI: 17469, 25711). We found no difference in D28 titres upon investigation of the extended time between second and third doses among the 16% of children vaccinated in Dandé. Data for participants at D28 reported an NANP IgG geometric mean of 324863 (197568-534176) compared with 248246 (220007-280110) for children receiving the third dose between 3-6 weeks after the second.

IgG titres induced by R21/Matrix-M™ vaccine at the D28 timepoint were higher in the 5-17 month age group compared with the older 18-36 month age group for all antigens when sites were combined (NANP: $p=0.0001$; C-term: $p=0.0005$; full length R21 $p=0.0001$; HBsAg $p<0.0001$) and for each site separately (Supplementary Tables S2a-d). After adjusting for site, sex, age at vaccination, and nutritional status, for CSP-antigens (NANP, C-Term and full length R21), but not for HBsAg, there was strong evidence that younger children (5-17 months) had a higher fold change than older children (18-36 months; $p<0.001$ for CSP-antigens and $p=0.929$ for HBsAg), and that children with a higher fold change were less likely have a malaria episode in the first year ($p=0.0002$, $p=0.0016$, $p=0.0227$ for NANP, C-term and R21 full length-specific IgG respectively, $p=0.5626$ for HBsAg-specific IgG; Supplementary Tables S3a-d).

At PV there is a positive trend between age and anti-NANP IgG PV ($p=0.06$; Figure 3a), while at D28 we observed a 1.8% reduction (95% CI 1.1-2.3%, $p<0.001$) in NANP-specific IgG GMT with each month of age at vaccination (Figure 3a). The same directionality at D28 is seen for C-term- and full length R21-specific IgG whereby the older a child was at first vaccination, the lower the CSP-specific IgG titre is after three doses of R21/Matrix-M™ vaccination (Figures 3b and c). For HBsAg-specific IgG, both PV and D28 titres were lower among older children. This corresponded to a 2.7% (95% CI 1.9, 3.5) decrease in D28 anti-HBsAg IgG titre for every extra month of age at vaccination, $p<0.001$; Figure 3d).

Twelve months after receiving the primary series, levels of antibodies waned but not to levels seen PV. IgG titres at B0 remained 4936, 1642, 4794 and 19-fold higher than PV for NANP, C-term, full length R21 and HBsAg respectively (geometric mean fold change for all sites, all age groups combined). We investigated the ability of the R21/Matrix-M™ booster dose to return NANP IgG to the levels seen at D28. This also varied by site and age group. The site level median percentage of NANP IgG titres at B28 relative to D28 titres ranged from 38-114% in 5-17-month-olds and between 40-90% in 18-36-month-olds (Figure 2 and Supplementary Table S4).

(16)

Notably, over 99.5% of measured individuals had PV anti-HBsAg IgG titres above the internationally recognised threshold for protection (16). We did not see any evidence to suggest that pre-vaccination HBsAg-specific IgG antibodies had any effect on R21/Matrix-M™-elicited CSP-specific IgG responses at any post-vaccination timepoint (Supplementary Figure S3). Nor did we see any evidence that pre-vaccination IgG to CSP epitopes had any impact on the ability of R21/Matrix-M™ vaccination to elicit high antibody titres to any vaccine antigens studied here. At all timepoints post-R21/Matrix-M™ vaccination, there was a positive correlation between all CSP-antigens (Supplementary Figure S3 and S4).

West African sites (Nanoro, Dandé and Bougouni) showed higher pre-vaccination NANP-specific IgG titres compared with East African sites (Supplementary Figure S2a). Participants from Dandé had higher pre-vaccination titres of C-term-specific IgG compared with other sites (Supplementary Figure S2b; other antigens shown in Supplementary Figure S2c-d).

D28 levels of both NANP- and C-Term-specific IgG levels associated with VE were evaluated as a correlate of VE per site, using the same method as previously used for Nanoro site in a Phase 2 R21/Matrix-M™ (13). The VE was 53.31% at the Bagamoyo site (lowest among all sites) and NANP and C-Term-specific IgG associated with that level of VE were 346441AU/mL and 212058AU/mL respectively in Bagamoyo. On the other hand, VE for Bougouni was 81.81% (highest among all sites) with 186751AU/mL (54% of the level of Bagamoyo) and 89024 AU/mL (42% of the level of Bagamoyo) for NANP and C-Term-specific IgG respectively (Supplementary Table S7).

Baseline parasitemia has no effect on anti-CSP immunogenicity post-primary vaccination series

A total of 221 individuals (4.95% 221/4464) were found to be *P.f* malaria blood film positive (>0 parasites/μl), on day of the first vaccination or up to 4 days prior. The mean age of blood film-positive children before first vaccination was 23.3 months, as compared to the mean age of blood film-negative children of 18.4 months ($p<0.001$). None of the malaria blood film-positive children were symptomatic and all were successfully vaccinated.

Associations between parasitemia at time of vaccination and pre-vaccination antibody titres, were investigated among the 2199 (95.3% - 2199/2308) children in the immunogenicity cohort who had a pre-vaccination blood film result, associations with post-vaccination antibody titres were investigated within the R21/Matrix-M™ arm only (95.5% - 1477/1546).

PV IgG specific to CSP-antigens were elevated among the individuals with pre-vaccination parasitemia ($p<0.001$; Figure 5a), with the effect consistent across sites. However, no associations were observed between level of parasitemia and magnitude of anti-CSP IgG (data not shown). D28 IgG specific to CSP-antigens showed no difference when baseline parasitemic and non-parasitemic individuals were compared (Figure 5b).

Faster decay of vaccine-specific antibodies in first six months after vaccination

At six months post primary series of three vaccinations (D180), IgG specific to all four antigens decreased from the D28 response. Across all sites, the geometric mean anti-NANP IgG antibody titre reduced by an average of 5.78% (95% CI 5.48-6.08) per(Figure 2 and Supplementary Table S5a). Over the subsequent six months to the day of booster dose (D180 to B0), the geometric mean anti-NANP IgG antibody titre reduced by an average of 2.72% (95% CI 2.52-2.91) per week(Supplementary Table S5a). At B0 titres were still maintained at a 4936-fold higher compared to PV ((95% CI 4454, 5470) $n=825$).

The rate of decay in anti-NANP IgG between D28 and D180 varied by site, with the percentage reduction per week ranging from 8.03% in Bagamoyo to 3.01% in Kilifi (all $p<0.001$). In the second six-month period (D180 to B0), the percentage change ranged from a decrease of 2.93% per week in Nanoro to a decrease of 2.08% per week in Dandé. All sites except Kilifi showed a significant difference in rate of decline between the two time periods (Supplementary Table S5a). The rate of decrease between the two time periods did not differ by sex, age at randomisation (5-17

months compared to 18-36 months), nutritional status, or whether a participant had a malaria episode within the first year (Supplementary Table S5a).

For other antigens, a similar pattern was seen, with significant variation in rates of antibody decay between sites, and with a greater decline in the first six-month period post-primary series compared with the second six months (Supplementary Table S5). Again, age, sex, nutritional status and whether an individual had none, or one or more episodes of clinical malaria, did not affect the rate of decline between the two time periods for any antigen, although there was some evidence anti HBsAg IgG antibodies declined faster in younger children (Supplementary Tables S5b-d).

Longevity of C-term-specific IgG associated with reduced rate of malaria incidence

We next sought to investigate the association between IgG antibody titres to the different CSP epitopes at D28, D180 and B0, to the rate of malaria incidence in the first year. Higher IgG antibody titres measured at D28 were associated with a lower rate of clinical malaria during the 12 months after the primary series for the CSP antigens; NANP; IRR: 0.63 (95% CI 0.44, 0.90), $p=0.0109$, C-term; IRR: 0.64 (95% CI 0.45, 0.91), $p=0.0126$ and full length R21; IRR: 0.68 (95% CI 0.48, 0.95), $p=0.0250$ (IRRs correspond to a 10 fold increase in antibody titre). (Figure 4 and Supplementary Table S6).

At D180, again, higher NANP-specific IgG was associated lower rates of clinical malaria with an IRR of 0.56 (95% CI 0.34-0.94, $p=0.0272$), C-term; IRR 0.41 (95% CI 0.23-0.70, $p=0.0014$), and anti-full length R21; IRR 0.48 (95% CI 0.27-0.84, $p=0.0100$) (Figure 4 and Supplementary Table S6).

At the B0 timepoint only increased C-term-specific IgG was associated with a reduced rate of malaria episodes; IRR 0.32 (95% CI 0.17-0.61, $p=0.0005$) (Figure 4 and Supplementary Table S6).

Discussion

The R21/Matrix-M™ malaria vaccine represents a landmark advance in the control of *P.f* infection, particularly among children living in high-burden areas of sub-Saharan Africa. The analysis of the immunological data from this Phase 3 trial demonstrates that R21/Matrix-M™ vaccination elicits a robust immune response to three different CSP-specific antigens and HBsAg. This response persists above pre-vaccination levels for at least a year and is successfully boosted by an additional dose after 12 months. The immune responses across different ages and geographical settings some show variation, as does the vaccine efficacy (14,17–20). Here, we highlight several important immunological observations from a Phase 3, multi-country vaccine trial, that expand our understanding of correlates of protection and the possible mechanisms by which R21/Matrix-M™ mediates efficacy.

Antibodies to CSP have been shown to be associated with vaccine efficacy in clinical trials of both RTS,S/AS01 (11,12) and R21/Matrix-M™ (8,13) however a clear threshold of IgG required for protection has not been established (21). Further investigation into immunologic correlates of protection using samples from RTS,S vaccine studies have identified some alternative single, or groups of candidates (22–29) however differences remain between populations, ages and studies.

We report differences between ages and sites with respect to R21/Matrix-M™ vaccine-induced IgG following the primary series. Notably, our longitudinal analyses reveal that, for all sites, vaccine-

specific IgG titres undergo a more rapid initial decline within the first six months following the primary series of vaccination compared to the subsequent six-month period, a pattern consistent with the biphasic decay kinetics observed for many vaccines (30). This early waning phase is presumed to reflect the contraction of short-lived plasmablasts and the transition to a more stable pool of long-lived plasma cells (31,32). A striking finding from our analysis was the heterogeneity in the magnitude and waning of vaccine-induced humoral responses across five sites across East (Tanzania, Kenya) and West (Burkina Faso, Mali) sub-Saharan African countries. Similar variations in immunological response between site and age groups have also been observed with the RTS,S/AS01 vaccine in which waning of anti-CSP antibody titres predicts duration of vaccine efficacy (ref White et al 2015 Lancet ID). While we see higher vaccine efficacy and higher anti-CSP IgG antibody titres in younger children we see no difference in antibody waning by age. The geographical variation in D28 anti-CSP IgG titres does not correlate with site level vaccine efficacy. This is exemplified by the finding that despite having less than half the GMT of NANP-specific IgG at D28 in both 5-17- and 18-36-month-olds compared to that of Bagamoyo, adjusted 12-month vaccine efficacy against time to first clinical malaria episode in Kilifi was comparable, differing by only 7 percentage points (60% in Bagamoyo vs 53% in Kilifi (8)). This raises the possibility that a cross-sectional measurement of IgG at one timepoint may not adequately represent the complexities of antibody abundance over an extended risk period.

After adjustment for NANP specific IgG titres, C-term-specific antibody avidity has been associated with RTS,S/AS01B VE in analysis of over a thousand infants living in Mali, Burkina Faso and Ghana (22). This is the first time that C-term-specific IgG titres have been found to be associated with efficacy of a CSP-vaccine (R21/Matrix-M™) in a malaria endemic area. Although we cannot adjust for NANP-specific IgG in our analysis due to the high correlation with C-term-specific IgG (Supplementary Figure S4) we see that the association between IgG titre and rate of malaria remains over a longer period of follow up for C-term-specific IgG than for NANP-specific IgG. These findings show that while there exists a clear association with R21/Matrix-M™ vaccine efficacy, the CSP-specific antibody magnitude after the primary series of vaccinations does not reveal generalisable thresholds to act as a single correlate of protection for all sites studied here. The lack of a consistent association between immune magnitude and efficacy underscores the necessity for multi-dimensional, systems serology, immune profiling when evaluating vaccine performance across diverse populations. Additional measures such as durability and breadth of immune response, use of AUC analysis, as well as the impact of host and environmental factors, in mediating vaccine efficacy will be important additions to further analysis.

The sensitivity of the MSD assay enabled quantification of CSP IgG prior to vaccination with R21/Matrix-M™. Indeed, other studies have used anti-sporozoite antibody titres as markers of recent exposure to malaria parasites and to estimate mosquito-parasite transmission (33,34). Here we report higher IgG specific to CSP constituents before vaccination when individuals have asymptomatic parasitemia suggesting a low, but measurable naturally acquired immune response to surface antigens of the sporozoite form of *P.f.* If used as a marker of natural exposure to sporozoites, the data reported here imply that previous antigenic exposure does not influence the ability of the R21/Matrix-M™ vaccine to elicit a robust response. This result is surprising given the immunomodulatory effects of asymptomatic parasitemia reported in other contexts, where chronic exposure can lead to immune exhaustion or hypo-responsiveness (35). In the case of viral vaccines, a variety of underlying mechanisms have been shown to result in pre-existing antibodies, derived from maternal-foetal transmission, by previous infection, or vaccination, blocking vaccine-induced protection for measles, adenovirus, and influenza vaccines (36). This is not always the case, specifically, in a SARS-Cov2 mRNA-vaccination study, participants with

pre-existing immunity mounted higher antibody responses faster, and reached higher antibody titres than individuals with no pre-existing antibodies (37).

The density of CSP on the surface of R21 has been reported to be sufficiently high to shield the hepatitis B core of the nanoparticle (38) from pre-existing antibodies. The finding that pre-existing antibodies to hepatitis B do not affect the ability of the R21/Matrix-M™ vaccine to elicit a robust CSP-specific response seems to support this hypothesis. Children enrolled to this clinical trial are very likely to have received Hepatitis B vaccine as part of the pentavalent vaccine administered via the Essential Programme of Immunisation given as a three-dose regimen prior to receiving R21/Matrix-M™ (39). Indeed, the majority of participants had HBsAg-specific IgG above the 10 mIU/mL WHO-specified protective level prior to R21/Matrix-M™ vaccination (Figure 3). Individuals given R21/Matrix-M™ had boosted responses to HBsAg which were then boosted again by the fourth dose given one year later. It will be interesting to follow the epidemiology of hepatitis B infection in areas rolling out R21/Matrix-M™ vaccine.

In summary, our findings reaffirm that R21/Matrix-M™ vaccine can generate robust, CSP-specific IgG responses. The durability of these responses and the ability of responses to be boosted by additional vaccine doses is influenced by factors that merit further study. The association between long-lived C-term-specific IgG and lower malaria incidence highlights a potentially critical target for enhancing vaccine efficacy. The absence of a relationship between pre-vaccination anti-CSP or HBsAg IgG titres and post-vaccination R21/Matrix-M™-induced immunogenicity, suggests that immune interference from either vaccination or prior malaria infection is unlikely to be a major factor in accounting for the age and geography-associated heterogeneity reported here. Together, these insights provide a compelling rationale for continued research to identify immunological markers of sustained protection, and to optimise the longevity of malaria vaccine-induced immunity in diverse populations. These findings will be critical to inform next-generation pre-erythrocytic vaccines and the need for repeated boosting.

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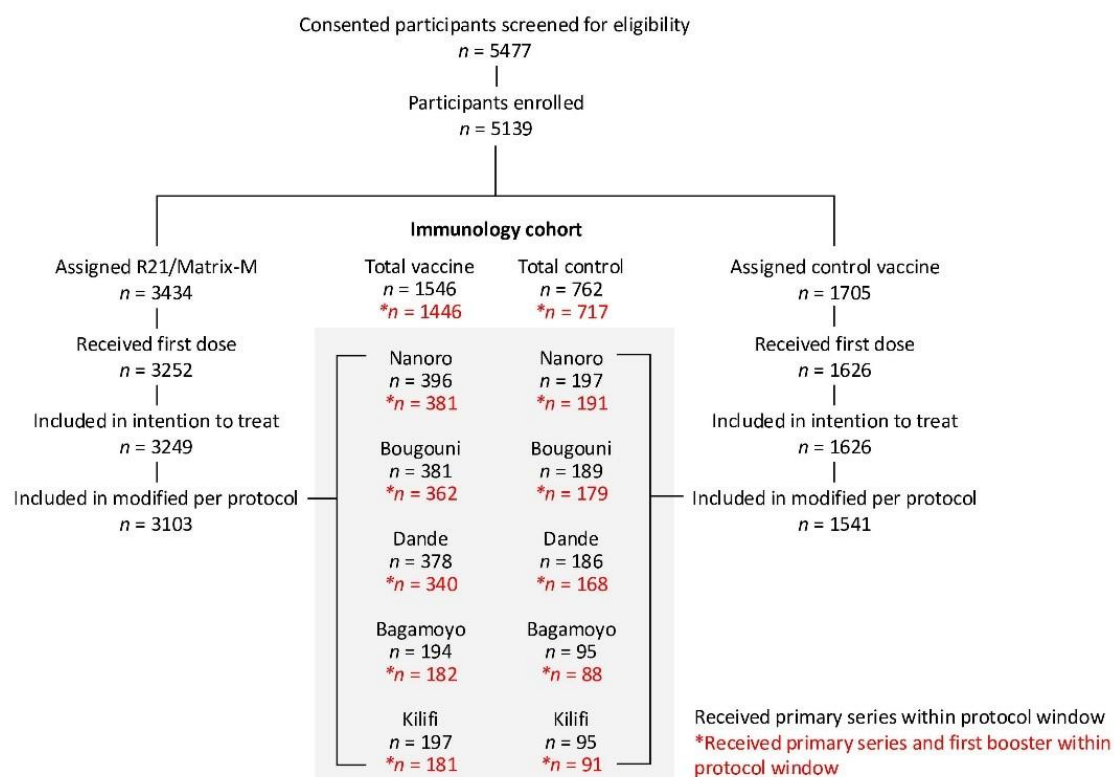


Figure 1: Consort diagram of enrolment and inclusion into immunogenecity cohort

Participants were aged 5–36 months on day of first vaccination (enrolment). 4800 participants ($\pm 4\%$) was the target enrolment number. The first 50% of participants enrolled at each site were included in the immunogenecity cohort and underwent additional safety and immunogenecity monitoring. The modified per protocol population comprises individuals who received the second vaccine dose 3–6 weeks after the first, the third dose 3–16 weeks after the second and the booster dose 365 ± 35 days after the third dose. Individuals are considered part of the modified per protocol immunogenecity cohort regardless of whether we have a valid immunology reading for them.

	All participants			Immunogenicity cohort	
Study Site	Age at randomisation, mean (sd)	Females, n/N (%)	VE* (95% CI)	Age at randomisation, mean (sd)	Females, n/N (%)
All Sites	19.0 (9.0)	2232/4644 (48.1)	72% (69, 76)	19.2 (9.0)	1119/2308 (48.5)
Nanoro	18.2 (9.1)	567/1185 (47.8)	73% (68, 77)	18.5 (9.3)	278/593 (46.9)
Bougouni	18.9 (9.2)	541/1154 (46.9)	80% (73, 85)	19.0 (9.0)	288/570 (50.5)
Dande	20.3 (8.6)	564/1165 (48.4)	75% (67, 81)	20.5 (8.9)	273/564 (48.4)
Bagamoyo	19.0 (8.9)	268/552 (48.6)	48% (19, 67)	19.1 (8.7)	147/289 (50.9)
Kilifi	18.5 (9.1)	292/588 (49.7)	59% (37, 73)	18.2 (8.7)	133/292 (45.5)

Table 1 - Demographic characteristics at screening for all study participants and those included in the immunogenicity cohort

Modified per-protocol population only, study arms combined. * VE (Vaccine efficacy) is efficacy of R21 against time to first malaria event from 14 days after completion of the primary series to 12 months following the primary series.

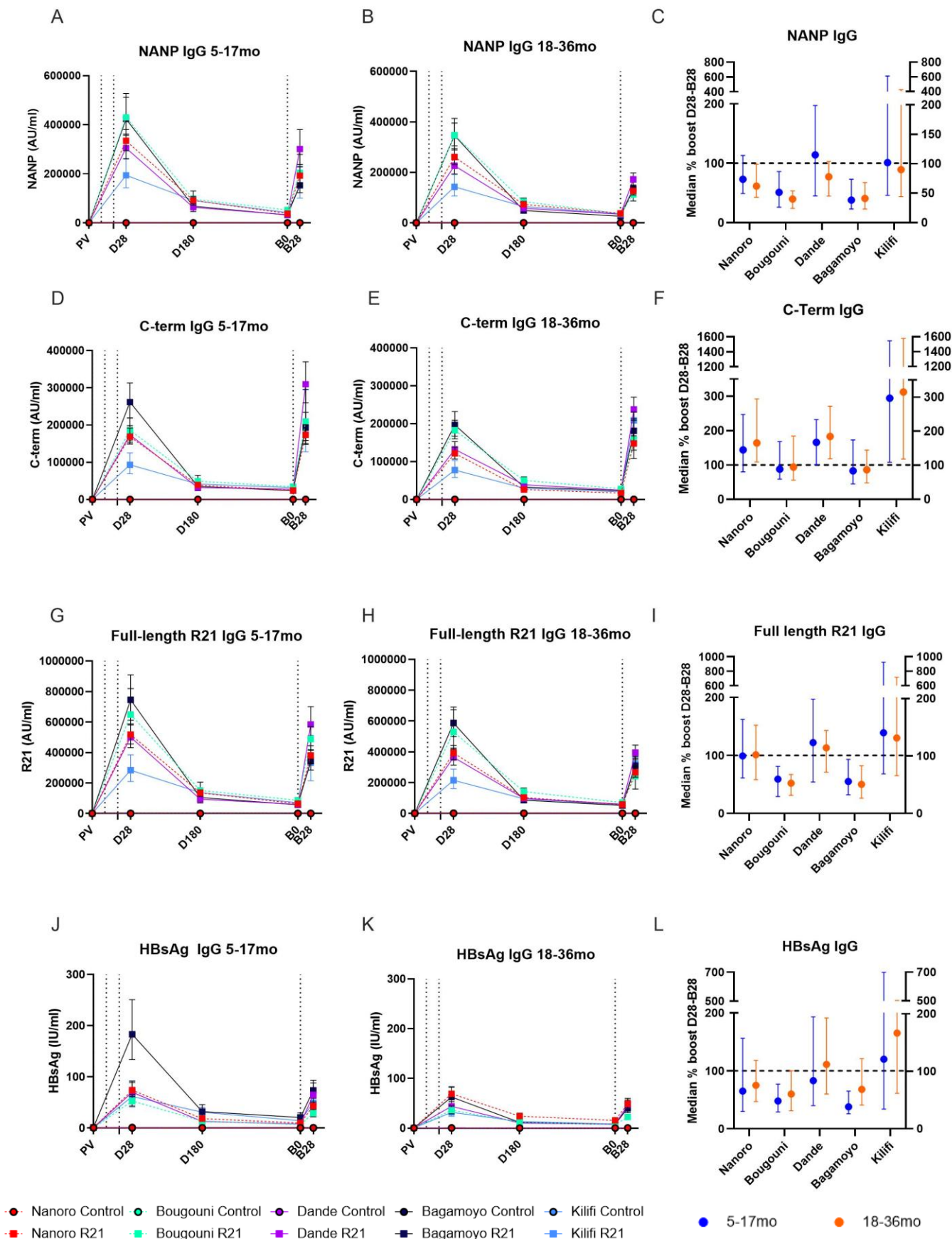


Figure 2 – Geometric mean, 95% confidence intervals for IgG antibody levels, and median percentage boosted between D28 and B28 for NANP, C-term, full-length R21 and HBsAg IgG by age group.

Geometric mean and 95% confidence intervals for IgG antibody levels specific to NANP (A and B), C-term (D and E), full length R21 (G and H), and hepatitis B surface antigen (HBsAg; J and K). All left-hand side plots show data for 5-17 month old participants (A, D, G and J) and all central plots show 18-36 month old participants (B, E, H and K). Data are shown split by vaccine and site. Vertical dashed lines indicate vaccination. Nanoro and Bougouni kinetics shown with dashed lines to indicate seasonal sites.

Right hand plots (C, F, I and L) show median percentage of D28 levels achieved by booster vaccination (B28) and interquartile range (IQR) for individuals for whom data for both timepoints was available. Horizontal dashed line represents 100%. Blue points are data for 5-17 month olds and orange for 18-36 month olds. PV – pre-vaccination, D28 – 28 days post primary series, D180 – 180 day/6 months post primary series, B0 – day of booster dose, B28 – 28 days post booster dose.

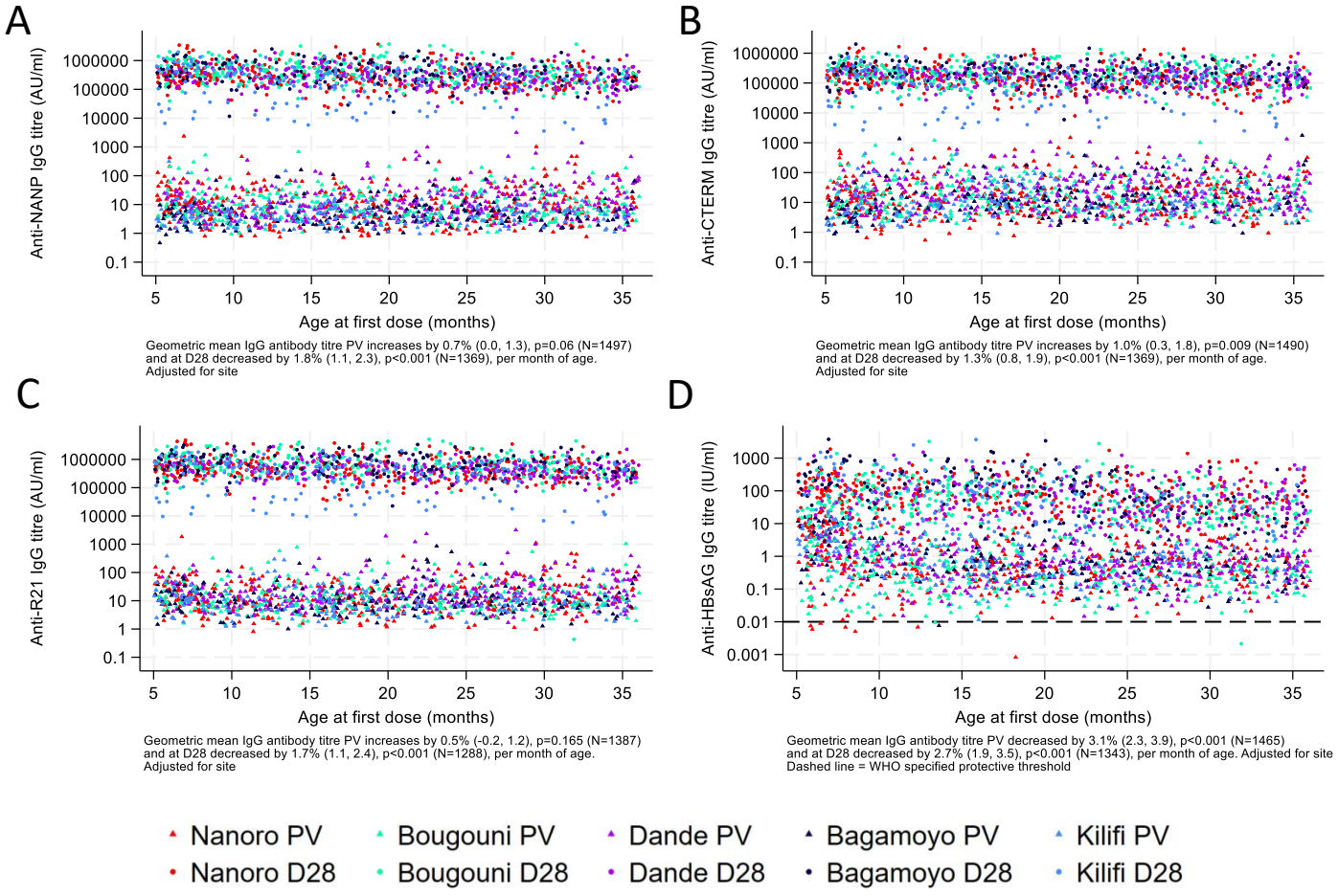


Figure 3 – Association between age at first vaccine dose and levels of IgG specific to A. NANP, B. C-Term, C. full length R21 and D. HBsAg IgG before vaccination (PV) and 28 days after (D28) primary series among participants in the R21/Matrix-M study arm, split by site.

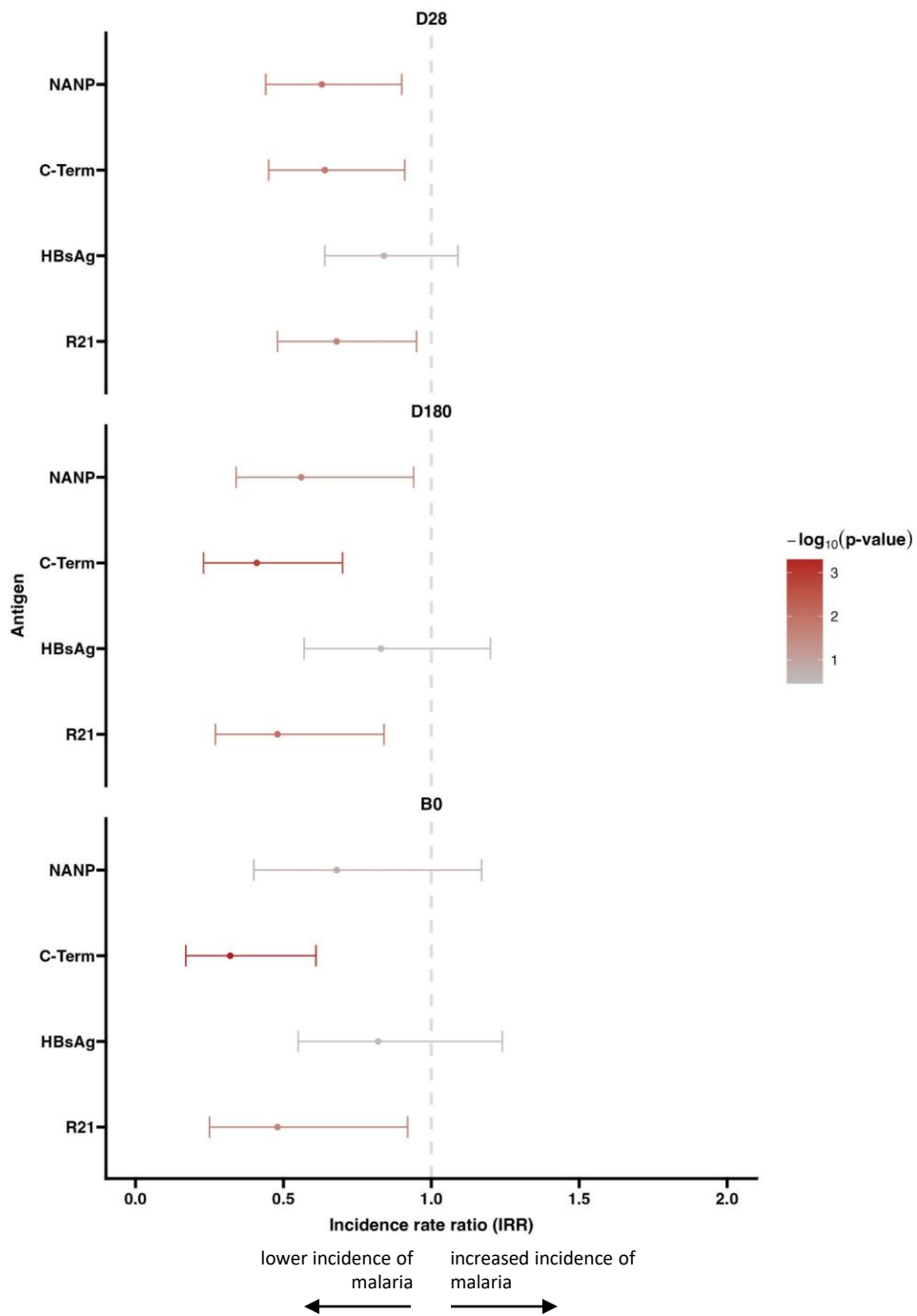


Figure 4 – Forest plot of incidence rate ratio of malaria episodes by antibody level.

Incidence Rate Ratio of clinical malaria per 10 fold increase in the IgG antibody titre in the R21/Matrix-M study arm measured at 28 (D28), 180 (D180) and 365 (B0) days after the third vaccine dose for four antigens during the first 12 months following the primary series. All data are adjusted for site. Point estimate shows IRR, whiskers show 95% confidence intervals. Raw data shown in table S6
IRR - incidence rate ratio.

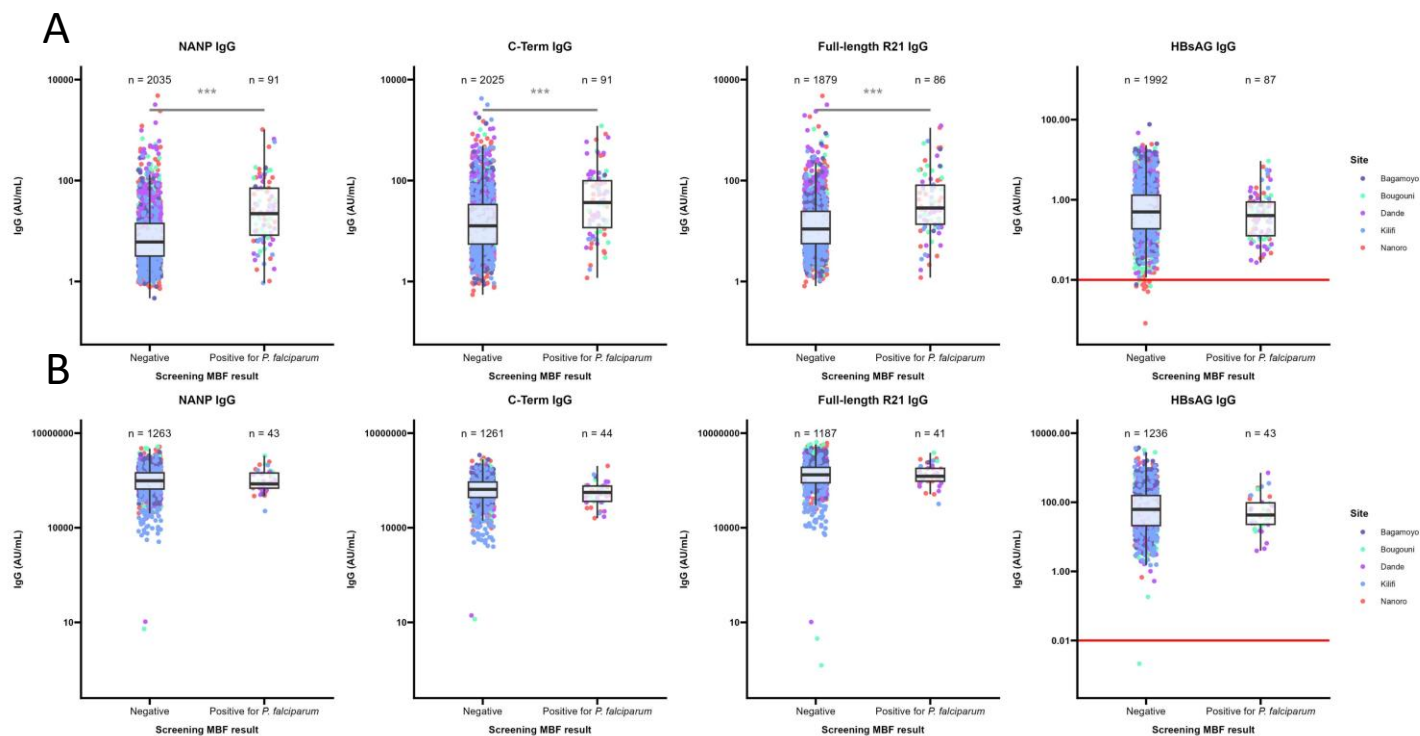


Figure 5 – Levels of IgG specific to NANP, C-Term, full length R21 and HBsAg (A) prior to vaccination among all participants whether randomised to received R21/Matrix-M or control vaccine, who were malaria blood-film (MBF) positive for *P.falciparum* parasites but asymptomatic (“Positive for *P.falciparum*”) or MBF negative (“Negative”) on the day of, or up to four days prior to first vaccination (“Screening MBF result”) (B) 28 days after primary series among participants in the R21/Matrix-M vaccination study arm only who were MBF positive for *P.falciparum* parasites but asymptomatic (“Positive for *P.falciparum*”) or MBF negative (“Negative”) on the day of, or up to four days prior to first vaccination *** $p < 0.001$.

Red lines on HBsAg shows the WHO-defined threshold for protection of 0.01IU/ml.

VAC078 (R21/MM Phase III) study design

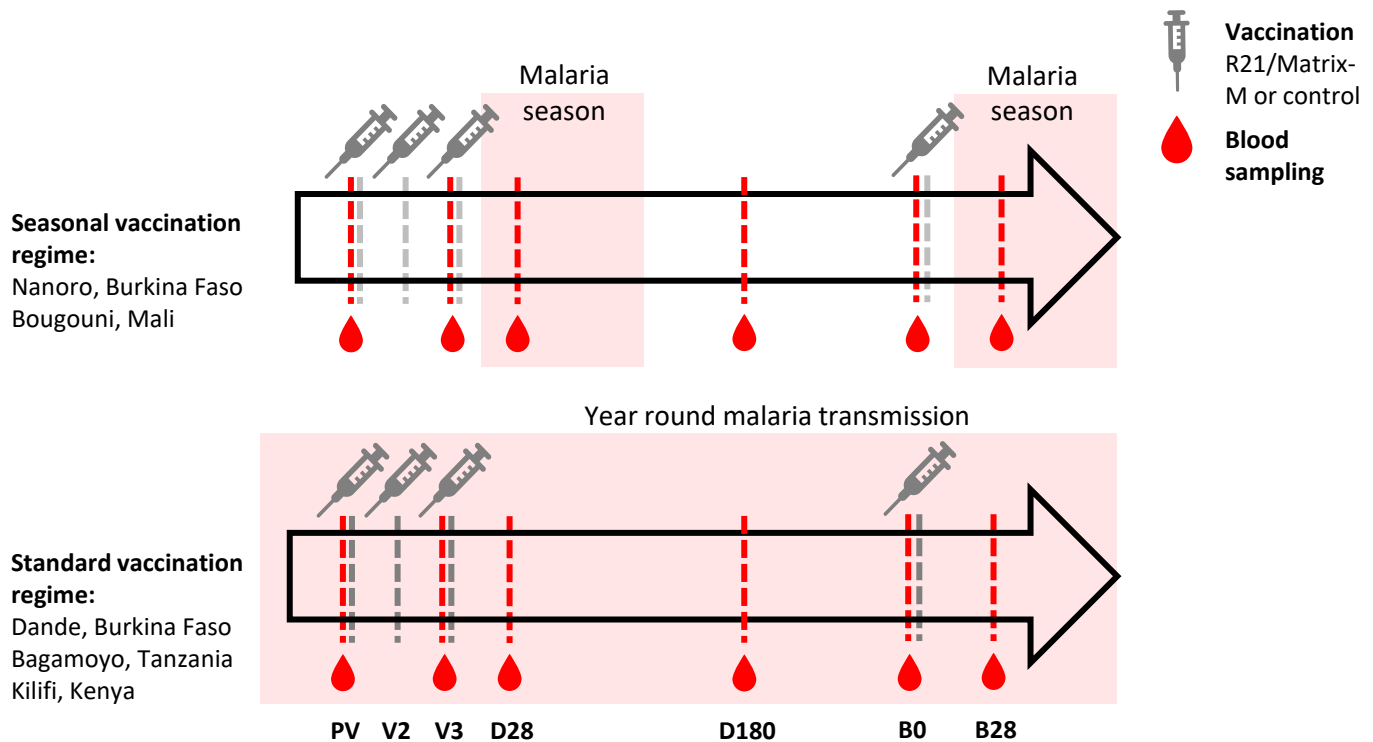


Figure S1 - Phase 3 clinical trial study overview.

All participants were given 3 vaccinations, 1 month apart, in the primary series followed by a first booster 12 months after the final primary series vaccination. Individuals were randomised 2:1 to receive either R21/Matrix-M™ or control vaccine. Blood samples were taken from the first 50% enrolled at each site (immunology cohort) at the following timepoints: at pre-vaccination (PV), 28 days post primary vaccination series (D28), 6 months/180 days (D180) post primary vaccination series, day of booster dose (pre-booster) (B0) and 28 days post booster dose (B28).

A

Site	Study group	MPP, n	MPP post-booster, n	PV	D28	D180	B0	B28
Nanoro	Control	197	191	194 (98.5%)	187 (94.9%)	39 (19.8%)	38 (19.9%)	38 (19.9%)
Bougouni		189	179	189 (100.0%)	179 (94.7%)	62 (32.8%)	36 (20.1%)	36 (20.1%)
Dande		186	168	172 (92.5%)	124 (66.7%)	36 (19.4%)	33 (19.6%)	39 (23.2%)
Bagamoyo		95	88	85 (89.5%)	79 (83.2%)	46 (48.4%)	47 (53.4%)	40 (45.5%)
Kilifi		95	91	95 (100.0%)	78 (82.1%)	69 (72.6%)	52 (57.1%)	17 (18.7%)
Nanoro	R21	396	381	388 (98.0%)	375 (94.7%)	251 (63.4%)	246 (64.6%)	244 (64.0%)
Bougouni		381	362	381 (100.0%)	350 (91.9%)	197 (51.7%)	181 (50.0%)	180 (49.7%)
Dande		378	340	348 (92.1%)	280 (74.1%)	175 (46.3%)	165 (48.5%)	127 (37.4%)
Bagamoyo		194	182	187 (96.4%)	180 (92.8%)	114 (58.8%)	116 (63.7%)	113 (62.1%)
Kilifi		197	181	193 (98.0%)	184 (93.4%)	147 (74.6%)	119 (65.7%)	107 (59.1%)

B

Site	Study group	MPP, n	MPP post-booster, n	PV	D28	D180	B0	B28
Nanoro	Control	197	191	192 (97.5%)	181 (91.9%)	40 (20.3%)	38 (19.9%)	39 (20.4%)
Bougouni		189	179	188 (99.5%)	174 (92.1%)	61 (32.3%)	36 (20.1%)	36 (20.1%)
Dande		186	168	171 (91.9%)	99 (53.2%)	36 (19.4%)	33 (19.6%)	40 (23.8%)
Bagamoyo		95	88	85 (89.5%)	80 (84.2%)	51 (53.7%)	47 (53.4%)	40 (45.5%)
Kilifi		95	91	95 (100.0%)	89 (93.7%)	69 (72.6%)	54 (59.3%)	17 (18.7%)
Nanoro	R21	396	381	388 (98.0%)	374 (94.4%)	252 (63.6%)	248 (65.1%)	239 (62.7%)
Bougouni		381	362	377 (99.0%)	351 (92.1%)	173 (45.4%)	131 (36.2%)	131 (36.2%)
Dande		378	340	347 (91.8%)	280 (74.1%)	178 (47.1%)	165 (48.5%)	127 (37.4%)
Bagamoyo		194	182	187 (96.4%)	180 (92.8%)	111 (57.2%)	114 (62.6%)	113 (62.1%)
Kilifi		197	181	191 (97.0%)	184 (93.4%)	151 (76.6%)	117 (64.6%)	107 (59.1%)

C

Site	Study group	MPP, n	MPP post-booster, n	PV	D28	D180	B0	B28
Nanoro	Control	197	191	190 (96.4%)	175 (88.8%)	39 (19.8%)	38 (19.9%)	37 (19.4%)
Bougouni		189	179	160 (84.7%)	172 (91.0%)	61 (32.3%)	36 (20.1%)	36 (20.1%)
Dande		186	168	151 (81.2%)	87 (46.8%)	16 (8.6%)	26 (15.5%)	40 (23.8%)
Bagamoyo		95	88	84 (88.4%)	81 (85.3%)	30 (31.6%)	38 (43.2%)	40 (45.5%)
Kilifi		95	91	95 (100.0%)	87 (91.6%)	69 (72.6%)	54 (59.3%)	17 (18.7%)
Nanoro	R21	396	381	386 (97.5%)	374 (94.4%)	250 (63.1%)	247 (64.8%)	243 (63.8%)
Bougouni		381	362	326 (85.6%)	273 (71.7%)	176 (46.2%)	131 (36.2%)	123 (34.0%)
Dande		378	340	298 (78.8%)	276 (73.0%)	177 (46.8%)	164 (48.2%)	129 (37.9%)
Bagamoyo		194	182	187 (96.4%)	181 (93.3%)	108 (55.7%)	113 (62.1%)	113 (62.1%)
Kilifi		197	181	190 (96.4%)	184 (93.4%)	149 (75.6%)	117 (64.6%)	107 (59.1%)

D

Site	Study group	MPP, n	MPP post-booster, n	PV	D28	D180	B0	B28
Nanoro	Control	197	191	193 (98.0%)	184 (93.4%)	40 (20.3%)	38 (19.9%)	39 (20.4%)
Bougouni		189	179	178 (94.2%)	173 (91.5%)	62 (32.8%)	37 (20.7%)	36 (20.1%)
Dande		186	168	168 (90.3%)	123 (66.1%)	36 (19.4%)	33 (19.6%)	40 (23.8%)
Bagamoyo		95	88	83 (87.4%)	81 (85.3%)	51 (53.7%)	47 (53.4%)	45 (51.1%)
Kilifi		95	91	95 (100.0%)	88 (92.6%)	70 (73.7%)	54 (59.3%)	17 (18.7%)
Nanoro	R21	396	381	383 (96.7%)	373 (94.2%)	250 (63.1%)	246 (64.6%)	243 (63.8%)
Bougouni		381	362	358 (94.0%)	346 (90.8%)	204 (53.5%)	184 (50.8%)	172 (47.5%)
Dande		378	340	345 (91.3%)	274 (72.5%)	177 (46.8%)	164 (48.2%)	129 (37.9%)
Bagamoyo		194	182	187 (96.4%)	175 (90.2%)	112 (57.7%)	111 (61.0%)	113 (62.1%)
Kilifi		197	181	192 (97.5%)	175 (88.8%)	156 (79.2%)	119 (65.7%)	107 (59.1%)

Table S1 - Number of samples analysed for A. NANP B. C-Term, C. full-length R21 and D. HBsAg-IgG antibodies at each timepoint, by site and study arm .

The MPP population post primary series was used to calculate the percentage data coverage for pre-vaccination (PV), D28 & D180 timepoints and the MPP population post-booster was used to calculate the percentage data coverage for B0 & B28. MPP – Modified per Protocol

Table S2A Geometric mean and 95% confidence intervals for NANP-specific IgG

Vaccine	Age (months)	PV	D28	D180	B0	B28
Nanoro						
Control	5-17	8 (6, 10) n=89	13 (9, 17) n=85	18 (6, 57) n=12	16 (6, 46) n=12	18 (6, 57) n=14
	18-36	9 (7, 12) n=105	22 (15, 31) n=102	17 (10, 27) n=27	10 (6, 17) n=26	19 (8, 45) n=24
R21	5-17	10 (8, 12) n=188	334279 (293743, 380408) n=181	91772 (79285, 106226) n=117	37722 (30882, 46078) n=115	192033 (161613, 228180) n=110
	18-36	9 (8, 11) n=200	260521 (230553, 294385) n=194	73434 (63365, 85103) n=134	37705 (30950, 45934) n=131	126272 (110266, 144601) n=134
Bougouni						
Control	5-17	9 (7, 11) n=91	11 (8, 15) n=85	11 (6, 19) n=27	13 (5, 33) n=12	16 (6, 40) n=11
	18-36	8 (7, 10) n=98	9 (6, 12) n=94	12 (6, 24) n=35	8 (5, 13) n=24	10 (6, 18) n=25
R21	5-17	8 (7, 10) n=187	430267 (361835, 511640) n=174	96185 (71213, 129914) n=84	52251 (43261, 63109) n=79	203839 (148936, 278981) n=77
	18-36	8 (7, 9) n=194	347443 (305357, 395329) n=176	84141 (72226, 98022) n=113	36342 (30942, 42683) n=102	112663 (86771, 146281) n=103
Dande						
Control	5-17	10 (7, 14) n=67	26 (15, 43) n=47	7 (4, 10) n=17	16 (8, 31) n=16	13 (6, 26) n=18
	18-36	10 (8, 12) n=105	39 (24, 64) n=77	9 (5, 18) n=19	18 (9, 38) n=17	14 (7, 30) n=21
R21	5-17	9 (7, 10) n=135	304525 (260076, 356571) n=98	63400 (45661, 88031) n=62	32641 (25547, 41704) n=59	300781 (237985, 380148) n=47
	18-36	14 (12, 17) n=213	226361 (193289, 265091) n=182	57819 (46609, 71725) n=113	34481 (29113, 40839) n=106	172246 (150209, 197516) n=80
Bagamoyo						
Control	5-17	5 (3, 6) n=42	36 (15, 91) n=36	8 (4, 18) n=24	10 (4, 24) n=20	7 (3, 16) n=19
	18-36	4 (3, 6) n=43	29 (15, 58) n=43	7 (4, 14) n=22	16 (7, 38) n=27	7 (2, 27) n=21
R21	5-17	4 (3, 5) n=79	423033 (339376, 527313) n=77	67924 (53409, 86383) n=51	31807 (24757, 40865) n=52	153244 (122835, 191181) n=51
	18-36	5 (4, 6) n=108	346082 (289388, 413881) n=103	48830 (38396, 62098) n=63	26249 (20592, 33460) n=64	132729 (107809, 163408) n=62
Kilifi						
Control	5-17	5 (4, 7) n=51	7 (5, 9) n=40	54 (35, 83) n=35	18 (11, 29) n=27	20 (13, 30) n=10
	18-36	6 (4, 8) n=44	9 (6, 13) n=38	85 (55, 129) n=34	18 (12, 29) n=25	17 (7, 40) n=7
R21	5-17	5 (4, 6) n=97	193817 (142939, 262805) n=92	91444 (75828, 110276) n=76	41540 (32805, 52601) n=57	152426 (100732, 230648) n=54
	18-36	5 (4, 6) n=96	142953 (106466, 191944) n=92	64961 (53567, 78778) n=71	34889 (27238, 44691) n=62	137047 (115024, 163287) n=53

Table S2B Geometric mean and 95% confidence intervals for C-term-specific IgG

Vaccine	Age (months)	PV	D28	D180	B0	B28
Nanoro						
Control	5-17	9 (7, 12) n=88	30 (23, 41) n=83	63 (26, 157) n=13	30 (15, 62) n=12	32 (15, 68) n=14
	18-36	14 (11, 19) n=104	26 (20, 34) n=98	26 (14, 52) n=27	23 (13, 40) n=26	30 (11, 82) n=25
R21	5-17	13 (11, 16) n=189	168970 (148755, 191933) n=179	38881 (33764, 44773) n=119	24012 (19898, 28976) n=115	173735 (150071, 201129) n=106
	18-36	16 (13, 19) n=199	121388 (106644, 138170) n=195	26150 (23198, 29478) n=133	16802 (14303, 19737) n=133	147769 (130186, 167727) n=133
Bougouni						
Control	5-17	13 (10, 17) n=91	17 (11, 24) n=83	14 (9, 19) n=27	15 (8, 30) n=12	19 (9, 40) n=11
	18-36	15 (12, 19) n=97	15 (11, 20) n=91	20 (12, 35) n=34	13 (9, 20) n=24	21 (14, 32) n=25
R21	5-17	15 (12, 18) n=184	186014 (157914, 219114) n=174	49329 (37488, 64909) n=76	34290 (28263, 41603) n=63	209526 (148158, 296314) n=63
	18-36	14 (12, 17) n=193	182659 (159780, 208814) n=177	50437 (42837, 59385) n=97	27826 (23168, 33420) n=68	157892 (108168, 230473) n=68
Dande						
Control	5-17	21 (15, 30) n=67	58 (29, 116) n=40	12 (7, 23) n=17	27 (13, 57) n=16	28 (14, 56) n=19
	18-36	27 (22, 34) n=104	49 (30, 80) n=59	13 (8, 21) n=19	22 (11, 41) n=17	18 (10, 33) n=21
R21	5-17	22 (18, 28) n=135	173864 (152341, 198429) n=98	31690 (23368, 42976) n=63	27664 (22820, 33537) n=59	309919 (259385, 370298) n=46
	18-36	25 (21, 30) n=212	132198 (114263, 152946) n=182	38795 (32189, 46756) n=115	24399 (20973, 28386) n=106	237372 (208714, 269966) n=81
Bagamoyo						
Control	5-17	11 (8, 15) n=42	63 (34, 118) n=38	28 (12, 66) n=24	16 (7, 39) n=20	18 (7, 46) n=19
	18-36	11 (7, 17) n=43	57 (30, 108) n=42	35 (15, 79) n=27	32 (14, 71) n=27	18 (6, 50) n=21
R21	5-17	9 (7, 13) n=80	261773 (218987, 312918) n=77	35162 (27811, 44454) n=49	23524 (19208, 28810) n=50	192756 (158684, 234144) n=51
	18-36	15 (11, 19) n=107	196767 (166855, 232041) n=103	32343 (27129, 38560) n=62	23026 (19813, 26761) n=64	181046 (153891, 212991) n=62
Kilifi						
Control	5-17	15 (10, 24) n=51	18 (12, 29) n=47	57 (40, 82) n=35	24 (14, 41) n=28	19 (9, 37) n=10
	18-36	14 (9, 22) n=44	16 (11, 24) n=42	65 (45, 93) n=34	17 (10, 28) n=26	13 (6, 29) n=7
R21	5-17	12 (9, 16) n=95	93073 (69227, 125132) n=92	41338 (35055, 48747) n=75	31928 (26176, 38944) n=57	194386 (128257, 294610) n=54
	18-36	14 (11, 18) n=96	77353 (58147, 102904) n=92	29900 (25235, 35429) n=76	20194 (16826, 24237) n=60	208778 (180453, 241550) n=53

Table S2C Geometric mean and 95% confidence intervals for full-length R21-specific IgG

Vaccine	Age (months)	PV	D28	D180	B0	B28
Nanoro						
Control	5-17	11 (9, 14) n=88	13 (9, 19) n=78	28 (10, 75) n=13	23 (9, 62) n=12	27 (12, 61) n=13
	18-36	14 (10, 19) n=102	36 (25, 53) n=97	31 (19, 49) n=26	23 (15, 37) n=26	49 (19, 126) n=24
R21	5-17	13 (11, 16) n=186	517031 (455256, 587188) n=180	135432 (118272, 155081) n=118	63381 (51839, 77493) n=115	380718 (326318, 444186) n=110
	18-36	13 (11, 16) n=200	391713 (347974, 440951) n=194	102819 (90216, 117184) n=132	56946 (46936, 69092) n=132	265902 (234517, 301486) n=133
Bougouni						
Control	5-17	12 (9, 15) n=78	13 (9, 20) n=82	16 (9, 26) n=27	24 (8, 76) n=12	19 (8, 50) n=11
	18-36	12 (10, 16) n=82	14 (10, 19) n=90	17 (9, 32) n=34	13 (9, 20) n=24	19 (12, 28) n=25
R21	5-17	11 (10, 14) n=156	648778 (513858, 819122) n=131	151505 (111760, 205385) n=75	85862 (70324, 104833) n=62	488281 (417857, 570574) n=56
	18-36	12 (10, 14) n=170	529078 (415860, 673119) n=142	140860 (120811, 164235) n=101	69673 (58334, 83217) n=69	241593 (157921, 369597) n=67
Dande						
Control	5-17	18 (13, 24) n=57	75 (34, 166) n=34	11 (6, 23) n=9	23 (12, 43) n=14	26 (13, 51) n=19
	18-36	17 (13, 21) n=94	165 (97, 281) n=53	9 (4, 21) n=7	20 (12, 36) n=12	20 (11, 38) n=21
R21	5-17	16 (13, 19) n=117	500213 (431250, 580204) n=97	93776 (68805, 127810) n=63	59913 (49030, 73211) n=59	584044 (486873, 700609) n=46
	18-36	26 (21, 31) n=181	363938 (312698, 423575) n=179	97393 (80466, 117880) n=114	59808 (51851, 68987) n=105	393757 (350359, 442531) n=83
Bagamoyo						
Control	5-17	12 (9, 17) n=41	64 (27, 150) n=38	50 (14, 179) n=15	19 (7, 56) n=18	16 (6, 43) n=19
	18-36	11 (8, 17) n=43	72 (35, 145) n=43	27 (11, 68) n=15	29 (11, 76) n=20	19 (6, 61) n=21
R21	5-17	10 (8, 13) n=80	745129 (610929, 908809) n=77	104762 (83435, 131540) n=47	56852 (45645, 70810) n=51	344017 (285588, 414400) n=51
	18-36	11 (9, 14) n=107	586178 (497904, 690103) n=104	85390 (72061, 101184) n=61	51265 (42894, 61268) n=62	308934 (265979, 358827) n=62
Kilifi						
Control	5-17	9 (7, 13) n=51	7 (6, 9) n=45	94 (67, 132) n=36	32 (19, 55) n=28	24 (15, 39) n=10
	18-36	9 (7, 12) n=44	9 (7, 13) n=42	137 (89, 211) n=33	27 (17, 44) n=26	26 (13, 53) n=7
R21	5-17	10 (8, 13) n=95	283993 (209749, 384517) n=92	137367 (116123, 162497) n=77	70676 (57744, 86503) n=57	331921 (214672, 513210) n=54
	18-36	9 (8, 11) n=95	214774 (160498, 287406) n=92	94279 (79311, 112071) n=72	54133 (43685, 67081) n=60	322554 (286443, 363218) n=53

Table S2D Geometric mean and 95% confidence intervals for Hepatitis B surface antigen-specific IgG

Vaccine	Age (months)	PV	D28	D180	B0	B28
Nanoro						
Control	5-17	0.7 (0.5, 1.1) n=88	0.5 (0.4, 0.7) n=83	0.3 (0.1, 0.6) n=13	0.3 (0.2, 0.5) n=12	0.4 (0.2, 0.7) n=14
	18-36	0.4 (0.3, 0.5) n=105	0.4 (0.3, 0.5) n=101	0.4 (0.3, 0.6) n=27	0.4 (0.2, 0.6) n=26	0.5 (0.3, 1.0) n=25
R21	5-17	0.5 (0.4, 0.7) n=183	73.8 (61.3, 89.0) n=180	18.3 (14.4, 23.1) n=117	9.8 (7.7, 12.4) n=111	43.0 (36.3, 50.9) n=109
	18-36	0.4 (0.4, 0.5) n=200	69.2 (58.0, 82.5) n=193	24.2 (19.4, 30.2) n=133	15.7 (12.4, 20.0) n=135	49.4 (40.8, 59.8) n=134
Bougouni						
Control	5-17	0.5 (0.3, 0.7) n=89	0.4 (0.2, 0.5) n=84	0.2 (0.1, 0.3) n=27	0.2 (0.1, 0.4) n=12	0.2 (0.1, 0.4) n=11
	18-36	0.3 (0.2, 0.3) n=89	0.3 (0.2, 0.3) n=89	0.2 (0.1, 0.3) n=35	0.2 (0.1, 0.2) n=25	0.2 (0.1, 0.3) n=25
R21	5-17	0.4 (0.3, 0.5) n=178	52.8 (42.9, 65.0) n=171	13.7 (10.6, 17.8) n=87	6.8 (5.4, 8.7) n=82	29.0 (21.9, 38.2) n=76
	18-36	0.3 (0.2, 0.3) n=180	36.2 (29.0, 45.3) n=175	12.0 (9.5, 15.2) n=117	7.0 (5.7, 8.6) n=102	22.4 (18.6, 27.0) n=96
Dande						
Control	5-17	0.7 (0.5, 1.0) n=65	0.7 (0.4, 1.0) n=46	0.4 (0.2, 0.8) n=17	0.5 (0.3, 0.9) n=16	0.7 (0.4, 1.3) n=19
	18-36	0.5 (0.4, 0.7) n=103	0.6 (0.4, 0.8) n=77	0.5 (0.3, 1.0) n=19	0.5 (0.3, 0.8) n=17	0.5 (0.3, 0.8) n=21
R21	5-17	0.8 (0.6, 1.1) n=134	70.0 (53.5, 91.6) n=98	12.8 (8.5, 19.4) n=62	7.9 (5.8, 10.8) n=59	64.7 (47.5, 88.1) n=47
	18-36	0.6 (0.5, 0.7) n=211	44.0 (36.2, 53.4) n=176	10.4 (7.8, 13.8) n=115	8.0 (6.3, 10.2) n=105	46.2 (37.6, 56.8) n=82
Bagamoyo						
Control	5-17	1.0 (0.6, 1.7) n=41	1.2 (0.7, 2.1) n=38	0.5 (0.2, 1.0) n=24	0.5 (0.2, 1.1) n=20	0.3 (0.1, 0.7) n=22
	18-36	0.5 (0.3, 0.6) n=42	0.8 (0.5, 1.3) n=43	0.4 (0.3, 0.6) n=27	0.4 (0.3, 0.6) n=27	0.6 (0.3, 1.2) n=23
R21	5-17	1.4 (1.0, 1.9) n=79	183.2 (133.8, 250.8) n=76	32.0 (22.5, 45.7) n=50	20.7 (14.6, 29.3) n=50	73.6 (58.0, 93.4) n=51
	18-36	0.4 (0.3, 0.5) n=108	62.6 (47.0, 83.5) n=99	11.6 (8.6, 15.7) n=62	7.9 (5.7, 10.9) n=61	40.1 (31.5, 51.2) n=62
Kilifi						
Control	5-17	0.7 (0.5, 1.1) n=51	0.4 (0.3, 0.6) n=47	0.3 (0.2, 0.4) n=36	0.2 (0.1, 0.4) n=28	0.2 (0.1, 0.6) n=10
	18-36	0.2 (0.2, 0.4) n=44	0.2 (0.2, 0.4) n=41	0.2 (0.1, 0.3) n=34	0.2 (0.1, 0.3) n=26	0.1 (0.0, 0.7) n=7
R21	5-17	0.9 (0.7, 1.2) n=96	61.6 (41.2, 92.2) n=88	30.9 (23.6, 40.4) n=77	14.4 (10.4, 20.0) n=57	49.6 (36.0, 68.5) n=54
	18-36	0.4 (0.3, 0.5) n=96	31.9 (23.9, 42.6) n=87	13.3 (10.3, 17.0) n=79	8.0 (6.2, 10.3) n=62	42.8 (33.4, 54.8) n=53

Table S2 Geometric mean and 95% confidence intervals for A. NANP-specific IgG B. C-term-specific IgG C. full length R21-specific IgG and D. Hepatitis B surface antigen (HBsAg) –specific IgG, by study arm, timepoint, study site, and age at first vaccine dose.

Statistical evidence for a difference in GMT IgG titres between age groups for anti-NANP IgG is as follows: Control: PV: p= 0.7037, D28: p= 0.0275, D180: p=0.3531, B0: p=0.5457, B28: p=0.8112 R21/Matrix-M: PV: p= 0.0916, D28: p= 0.0001, D180: p=0.0197, B0: p=0.1063, B28: p<0.0001.

Statistical evidence for a difference in GMT IgG titres between age groups for C-term IgG is as follows: Control: PV: p= 0.0307, D28: p= 0.2455, D180: p=0.6422, B0: p=0.7321, B28: p=0.6752 R21/Matrix-M: PV: p= 0.0343, D28: p= 0.0005, D180: p=0.1018, B0: p=0.0004, B28: p=0.0817

Statistical evidence for a difference in GMT IgG titres between age groups for full-length R21 IgG is as follows: Control: PV: p= 0.6625, D28: p<0.0001, D180: p=0.9393, B0: p=0.3787, B28: p=0.4331 R21/Matrix-M: PV: p= 0.0437, D28: p= 0.0001, D180: p=0.0397, B0: p=0.0887, B28: p=0.0001

Statistical evidence for a difference in GMT IgG titres between age groups for HBsAg is as follows: Control: PV: p<0.0001, D28: p= 0.0159, D180: p=0.8593, B0: p=0.3844, B28: p=0.4962 R21/Matrix-M: PV: p<0.0001, D28: p<0.0001, D180: p=0.0014, B0: p=0.3595, B28: p=0.0214

The MPP population post primary series was used to calculate the GMTs pre-vaccination (PV), D28 & D180 timepoints and the MPP population post-booster was used to calculate the percentage data coverage for B0 & B28. MPP – Modified per Protocol

A

NANP IgG

	Geometric mean fold change NANP IgG between PV and D28 (95% CI) and p value among R21 vaccinated individuals	p-value	Geometric mean fold change NANP IgG between PV and D28 (95% CI) and p value among control vaccinated individuals	p-value
All	37295 (34260, 40600) n=1363		2 (2, 2) n=647	
Site				
Nanoro	31690 (26983, 37217) n=373		2 (2, 3) n=187	
Bougoun	48593 (41644, 56701) n=350		1 (1, 1) n=179	
Dande	21193 (17469, 25711) n=279		3 (2, 5) n=124	
Bagamoyo	80495 (66164, 97932) n=179		7 (4, 12) n=79	
Kilifi	34939 (27361, 44615) n=182	<0.0001	1 (1, 2) n=78	<0.0001
Sex				
Male	37373 (32986, 42343) n=689		2 (2, 3) n=337	
Female	37216 (33168, 41759) n=674	0.7502	2 (2, 3) n=310	0.8242
Age at randomisation				
5-17 mo	47662 (40927, 55504) n=394		2 (2, 3) n=182	
18-36 mo	41288 (35728, 47712) n=512	0.0002	1 (1, 2) n=251	0.4523
Nutritional Status				
Normal WAZ (>-2)	38922 (35523, 42645) n=1141		2 (2, 3) n=536	
Underweight (<-2)	30011 (23919, 37654) n=221	0.182	1 (1, 3) n=110	0.8108
Clinical malaria within first year				
No episodes	40396 (36869, 44261) n=1186		2 (2, 2) n=402	
At least 1 episode	21839 (17605, 27091) n=177	0.0002	2 (2, 3) n=245	0.0829

C

full-length R21 IgG

	Geometric mean fold change full-length R21 IgG between PV and D28 (95% CI) and p value among R21 vaccinated individuals	p-value	Geometric mean fold change full-length R21 IgG between PV and D28 (95% CI) and p value among control vaccinated individuals	p-value
All	33873 (30679, 36959) n=1201		2 (2, 2) n=557	
Site				
Nanoro	34010 (29298, 39479) n=370		2 (1, 2) n=171	
Bougoun	51043 (41297, 63089) n=240		1 (1, 1) n=146	
Dande	17470 (13950, 21878) n=232		6 (4, 11) n=73	
Bagamoyo	59105 (48318, 72301) n=180		6 (3, 10) n=80	
Kilifi	25105 (19378, 32524) n=179	<0.0001	1 (1, 1) n=87	<0.0001
Sex				
Male	34273 (29739, 39498) n=611		2 (2, 2) n=288	
Female	33062 (29320, 37282) n=590	0.7291	2 (2, 3) n=269	0.6358
Age at randomisation				
5-17 mo	42367 (36188, 49601) n=344		1 (1, 2) n=162	
18-36 mo	35860 (30583, 42047) n=449	<0.0001	2 (2, 3) n=216	0.0291
Nutritional Status				
Normal WAZ (>-2)	34524 (31156, 38255) n=999		2 (2, 2) n=454	
Underweight (<-2)	29797 (23832, 37253) n=201	0.6705	1 (1, 4) n=102	0.5372
Clinical malaria within first year				
No episodes	35574 (32117, 39403) n=1034		2 (1, 2) n=347	
At least 1 episode	23965 (19350, 29680) n=167	0.0227	2 (2, 3) n=210	0.0856

B

C-Term IgG

	Geometric mean fold change C-Term IgG between PV and D28 (95% CI) and p value among R21 vaccinated individuals	p-value	Geometric mean fold change C-Term IgG between PV and D28 (95% CI) and p value among control vaccinated individuals	p-value
All	9619 (8962, 10757) n=1355		2 (2, 2) n=620	
Site				
Nanoro	10187 (8636, 12018) n=372		3 (2, 3) n=179	
Bougoun	13159 (11142, 15541) n=347		1 (1, 1) n=173	
Dande	6769 (4763, 6988) n=277		2 (1, 3) n=99	
Bagamoyo	17750 (13924, 22626) n=179		6 (4, 8) n=80	
Kilifi	8508 (4837, 8757) n=180	<0.0001	1 (1, 1) n=89	<0.0001
Sex				
Male	10021 (8768, 11453) n=683		2 (2, 2) n=321	
Female	9617 (8491, 10893) n=672	0.4843	2 (2, 2) n=299	0.6815
Age at randomisation				
5-17 mo	14873 (12667, 17462) n=390		3 (2, 3) n=181	
18-36 mo	8425 (7213, 9842) n=507	0.0002	1 (1, 2) n=240	0.0032
Nutritional Status				
Normal WAZ (>-2)	10252 (9275, 11333) n=1132		2 (2, 2) n=511	
Underweight (<-2)	7962 (6387, 9926) n=222	0.1623	1 (1, 3) n=108	0.6318
Clinical malaria within first year				
No episodes	10485 (9497, 11576) n=1181		2 (2, 2) n=389	
At least 1 episode	6287 (5033, 7854) n=174	0.0016	1 (1, 2) n=231	0.7752

D

HBsAg IgG

	Geometric mean fold change HBsAg IgG between PV and D28 (95% CI) and p value among R21 vaccinated individuals	p-value	Geometric mean fold change HBsAg IgG between PV and D28 (95% CI) and p value among control vaccinated individuals	p-value
All	117 (108, 127) n=1307		1 (1, 1) n=635	
Site				
Nanoro	149 (129, 172) n=366		1 (1, 1) n=183	
Bougoun	145 (122, 174) n=325		1 (1, 1) n=166	
Dande	78 (67, 92) n=270		1 (1, 1) n=119	
Bagamoyo	140 (110, 178) n=174		1 (1, 2) n=79	
Kilifi	73 (56, 95) n=172	<0.0001	1 (1, 1) n=88	0.0018
Sex				
Male	121 (107, 136) n=663		1 (1, 1) n=330	
Female	113 (100, 127) n=644	0.6327	1 (1, 1) n=305	0.9661
Age at randomisation				
5-17 mo	87 (73, 103) n=375		1 (0, 1) n=183	
18-36 mo	178 (155, 205) n=497	0.9239	1 (1, 1) n=244	<0.0001
Nutritional Status				
Normal WAZ (>-2)	111 (101, 122) n=1092		1 (1, 1) n=527	
Underweight (<-2)	152 (123, 187) n=214	0.0046	1 (1, 1) n=107	0.8589
Clinical malaria within first year				
No episodes	115 (105, 126) n=1133		1 (1, 1) n=400	
At least 1 episode	132 (106, 163) n=174	0.5624	1 (1, 1) n=235	0.3392

Table S3 – Geometric mean and 95% confidence intervals of fold change between pre-vaccination (PV) and 28 days post primary series (D28) A. NANP B. C-Term D. full length R21 and D. HBsAg-specific IgG.

By site, sex, age at randomisation (5-17m vs 18-36m), nutritional status (underweight vs normal weight) and number of malaria cases (0 vs 1+), among participants in the R21/Matrix-M™ and control vaccine study arms. P-values are from linear regression of log transformed fold-change, adjusting for site, sex, age group at randomisation and nutritional status.

		NANP		C-Term		Full length R21		HBsAG	
Age at randomisation	Site	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)
5-17 months	Nanoro	110	73 (50, 113)	106	144 (80, 247)	110	99 (61, 162)	109	65 (30, 156)
	Bougouni	76	51 (26, 86)	62	88 (59, 168)	34	59 (29, 81)	74	48 (29, 77)
	Dande	43	114 (45, 197)	42	166 (101, 232)	42	122 (54, 197)	43	83 (40, 193)
	Bagamoyo	50	38 (23, 73)	50	83 (45, 173)	50	55 (32, 93)	50	38 (26, 65)
	Kilifi	53	101 (46, 612)	53	294 (108, 1547)	53	139 (68, 925)	49	120 (34, 699)
18-36 months	Nanoro	133	62 (43, 99)	132	164 (109, 292)	131	101 (58, 152)	131	75 (47, 119)
	Bougouni	100	40 (24, 54)	68	94 (56, 184)	48	52 (31, 67)	94	60 (31, 100)
	Dande	75	78 (45, 104)	75	183 (118, 271)	75	113 (71, 143)	71	111 (60, 191)
	Bagamoyo	61	41 (23, 68)	62	86 (48, 143)	62	50 (26, 82)	57	68 (41, 121)
	Kilifi	52	90 (44, 425)	52	312 (117, 1578)	52	130 (65, 716)	49	165 (61, 505)

Table S4- Median percent boost with interquartile range between D28 and B28 per site, age group and antigen for all participants with valid immunology measurements at both timepoints.

	N	Percentage reduction in geometric mean per week* % (95% CI) p-value		Does change in antibody concentration differ between periods p-value	Test for interaction
		One month post primary series to six months post primary series (D28-D180)	Six months post primary series to pre-booster (D180-B0)		
All		1504	5.78 (5.48, 6.08) <0.001	2.72 (2.52, 2.91) <0.001	<0.001
Site					
Nanoro	390	5.12 (4.58, 5.66) <0.001	2.93 (2.59, 3.27) <0.001	<0.001	<0.001
Bougouni	381	6.34 (5.76, 6.92) <0.001	2.81 (2.39, 3.21) <0.001	<0.001	
Dande	350	6.44 (5.82, 7.07) <0.001	2.08 (1.60, 2.56) <0.001	<0.001	
Bagamoyo	188	8.03 (7.26, 8.79) <0.001	2.71 (2.20, 3.22) <0.001	<0.001	
Kilifi	195	3.01 (2.27, 3.75) <0.001	2.69 (2.21, 3.16) <0.001	0.524	
Sex					
Male	774	5.63 (5.21, 6.04) <0.001	2.63 (2.35, 2.90) <0.001	<0.001	0.404
Female	730	5.94 (5.52, 6.36) <0.001	2.80 (2.53, 3.07) <0.001	<0.001	
Age at vaccination					
5-17 months	690	5.79 (5.35, 6.24) <0.001	2.94 (2.65, 3.23) <0.001	<0.001	0.937
18-36 months	814	5.77 (5.37, 6.16) <0.001	2.54 (2.28, 2.80) <0.001	<0.001	
Nutritional status					
Normal WAZ (> -2)	1261	5.78 (5.45, 6.10) <0.001	2.69 (2.47, 2.90) <0.001	<0.001	0.741
Underweight (< -2)	242	5.82 (5.08, 6.56) <0.001	2.89 (2.40, 3.38) <0.001	<0.001	
Clinical malaria within first year					
No episodes	1315	5.86 (5.55, 6.18) <0.001	2.72 (2.52, 2.93) <0.001	<0.001	0.223
At least 1 episode	189	5.23 (4.40, 6.05) <0.001	2.67 (2.13, 3.20) <0.001	<0.001	

Table S5A - Change in geometric mean NANP-specific IgG antibody titre between D28 and B0

By site, sex, age at randomisation (5-17m vs 18-36m), nutritional status (underweight vs normal weight) and number of malaria cases (0 vs 1+), among participants in the R21/Matrix-M™ study arm. *adjusted for site, sex, age group (5-17m vs 18-36m), nutritional status and number of SMC rounds in 2021.

	N	Percentage reduction in geometric mean per week* % (95% CI) p-value		Does change in antibody concentration differ between periods	Test for interaction
		One month post primary series to six months post primary series (D28-D180)	Six months post primary series to pre-booster (D180-B0)		
					p-value
All	1504	6.07 (5.79, 6.34) <0.001	1.59 (1.39, 1.78) <0.001	<0.001	-
Site					
Nanoro	390	6.19 (5.69, 6.69) <0.001	1.82 (1.49, 2.15) <0.001	<0.001	<0.001
Bougouni	381	6.01 (5.44, 6.57) <0.001	1.80 (1.32, 2.28) <0.001	<0.001	
Dande	350	6.29 (5.70, 6.87) <0.001	1.14 (0.68, 1.59) <0.001	<0.001	
Bagamoyo	188	8.18 (7.46, 8.90) <0.001	1.45 (0.94, 1.95) <0.001	<0.001	
Kilifi	195	3.55 (2.86, 4.23) <0.001	1.28 (0.81, 1.75) <0.001	<0.001	
Sex					
Male	774	6.16 (5.77, 6.55) <0.001	1.43 (1.15, 1.71) <0.001	<0.001	0.589
Female	730	5.97 (5.58, 6.36) <0.001	1.74 (1.46, 2.01) <0.001	<0.001	
Age at vaccination					
5-17 months	690	6.17 (5.76, 6.58) <0.001	1.45 (1.16, 1.74) <0.001	<0.001	0.421
18-36 months	814	5.98 (5.61, 6.35) <0.001	1.70 (1.44, 1.96) <0.001	<0.001	
Nutritional status					
Normal WAZ (> -2)	1261	6.06 (5.76, 6.36) <0.001	1.49 (1.28, 1.70) <0.001	<0.001	0.918
Underweight (< -2)	242	6.08 (5.39, 6.76) <0.001	2.11 (1.62, 2.60) <0.001	<0.001	
Clinical malaria within first year					
No episodes	1315	6.06 (5.77, 6.36) <0.001	1.56 (1.35, 1.77) <0.001	<0.001	0.903
At least 1 episode	189	6.10 (5.34, 6.86) <0.001	1.75 (1.22, 2.27) <0.001	<0.001	

Table S5b- Change in geometric mean C-Term-specific IgG antibody titre between D28 and pre-booster B0)

By site, sex, age at randomisation (5-17m vs 18-36m), nutritional status (underweight vs normal weight) and number of malaria cases (0 vs 1+), among participants in the R21/Matrix-M™ study arm. *adjusted for site, sex, age group (5-17m vs 18-36m), nutritional status and number of SMC rounds in 2021.

	N	Percentage reduction in geometric mean per week* % (95% CI) p-value		Does change in antibody concentration differ between periods p-value	Test for interaction
		One month post primary series to six months post primary series (D28-D180)	Six months post primary series to pre-booster (D180-B0)		
All		1501	5.90 (5.59, 6.21) <0.001	2.30 (2.10, 2.50) <0.001	<0.001
Site					
Nanoro	390	5.45 (4.90, 5.99) <0.001	2.58 (2.24, 2.92) <0.001	<0.001	<0.001
Bougouni	379	6.25 (5.60, 6.90) <0.001	2.29 (1.80, 2.79) <0.001	<0.001	
Dande	349	6.56 (5.92, 7.19) <0.001	1.67 (1.19, 2.15) <0.001	<0.001	
Bagamoyo	188	8.24 (7.46, 9.01) <0.001	2.23 (1.69, 2.76) <0.001	<0.001	
Kilifi	195	3.16 (2.41, 3.90) <0.001	2.31 (1.82, 2.80) <0.001	0.099	
Sex					
Male	773	5.78 (5.35, 6.21) <0.001	2.13 (1.84, 2.42) <0.001	<0.001	0.421
Female	728	6.02 (5.59, 6.46) <0.001	2.46 (2.17, 2.74) <0.001	<0.001	
Age at vaccination					
5-17 months	688	6.01 (5.55, 6.47) <0.001	2.45 (2.14, 2.75) <0.001	<0.001	0.550
18-36 months	813	5.80 (5.39, 6.21) <0.001	2.18 (1.91, 2.45) <0.001	<0.001	
Nutritional status					
Normal WAZ (> -2)	1259	5.89 (5.55, 6.23) <0.001	2.23 (2.01, 2.45) <0.001	<0.001	0.778
Underweight (< -2)	241	5.95 (5.19, 6.70) <0.001	2.69 (2.18, 3.19) <0.001	<0.001	
Clinical malaria within first year					
No episodes	1312	5.94 (5.61, 6.27) <0.001	2.30 (2.09, 2.52) <0.001	<0.001	0.582
At least 1 episode	189	5.65 (4.80, 6.49) <0.001	2.27 (1.72, 2.82) <0.001	<0.001	

Table S5c - Change in geometric mean full length R21-specific IgG antibody titre between D28 and pre-booster (B0)

By site, sex, age at randomisation (5-17m vs 18-36m), nutritional status (underweight vs normal weight) and number of malaria cases (0 vs 1+), among participants in the R21/Matrix-M study arm. *adjusted for site, sex, age group (5-17m vs 18-36m), nutritional status and number of SMC rounds in 2021.

	N	Percentage reduction in geometric mean per week* % (95% CI) p-value		Does change in antibody concentration differ between periods p-value	Test for interaction
		One month post primary series to six months post primary series (D28-D180)	Six months post primary series to pre-booster (D180-B0)		
All	1488	5.67 (5.37, 5.98) <0.001	1.78 (1.60, 1.96) <0.001	<0.001	-
Site					
Nanoro	390	5.10 (4.54, 5.66) <0.001	2.05 (1.73, 2.37) <0.001	<0.001	<0.001
Bougouni	369	5.71 (5.11, 6.30) <0.001	1.98 (1.60, 2.35) <0.001	<0.001	
Dande	346	7.10 (6.46, 7.74) <0.001	0.70 (0.25, 1.15) 0.003	<0.001	
Bagamoyo	188	7.18 (6.37, 7.99) <0.001	1.62 (1.13, 2.11) <0.001	<0.001	
Kilifi	195	3.29 (2.52, 4.05) <0.001	2.18 (1.74, 2.62) <0.001	0.028	
Sex					
Male	766	5.57 (5.14, 6.00) <0.001	1.76 (1.50, 2.02) <0.001	<0.001	0.637
Female	722	5.78 (5.35, 6.21) <0.001	1.80 (1.54, 2.06) <0.001	<0.001	
Age at vaccination					
5-17 months	679	6.01 (5.56, 6.46) <0.001	2.17 (1.90, 2.45) <0.001	<0.001	0.052
18-36 months	809	5.41 (5.00, 5.82) <0.001	1.47 (1.22, 1.71) <0.001	<0.001	
Nutritional status					
Normal WAZ (> -2)	1247	5.61 (5.28, 5.94) <0.001	1.72 (1.53, 1.92) <0.001	<0.001	0.404
Underweight (< -2)	240	5.99 (5.24, 6.73) <0.001	2.09 (1.63, 2.54) <0.001	<0.001	
Clinical malaria within first year					
No episodes	1299	5.72 (5.40, 6.05) <0.001	1.78 (1.58, 1.97) <0.001	<0.001	0.259
At least 1 episode	189	5.35 (4.50, 6.20) <0.001	1.81 (1.31, 2.31) <0.001	<0.001	

Table S5d- Change in geometric mean HBsAg-specific IgG antibody titre between D28 and pre-booster (B0)

By site, sex, age at randomisation (5-17m vs 18-36m), nutritional status (underweight vs normal weight) and number of malaria cases (0 vs 1+), among participants in the R21/Matrix-M™ study arm. *adjusted for site, sex, age group (5-17m vs 18-36m), nutritional status and number of SMC rounds in 2021.

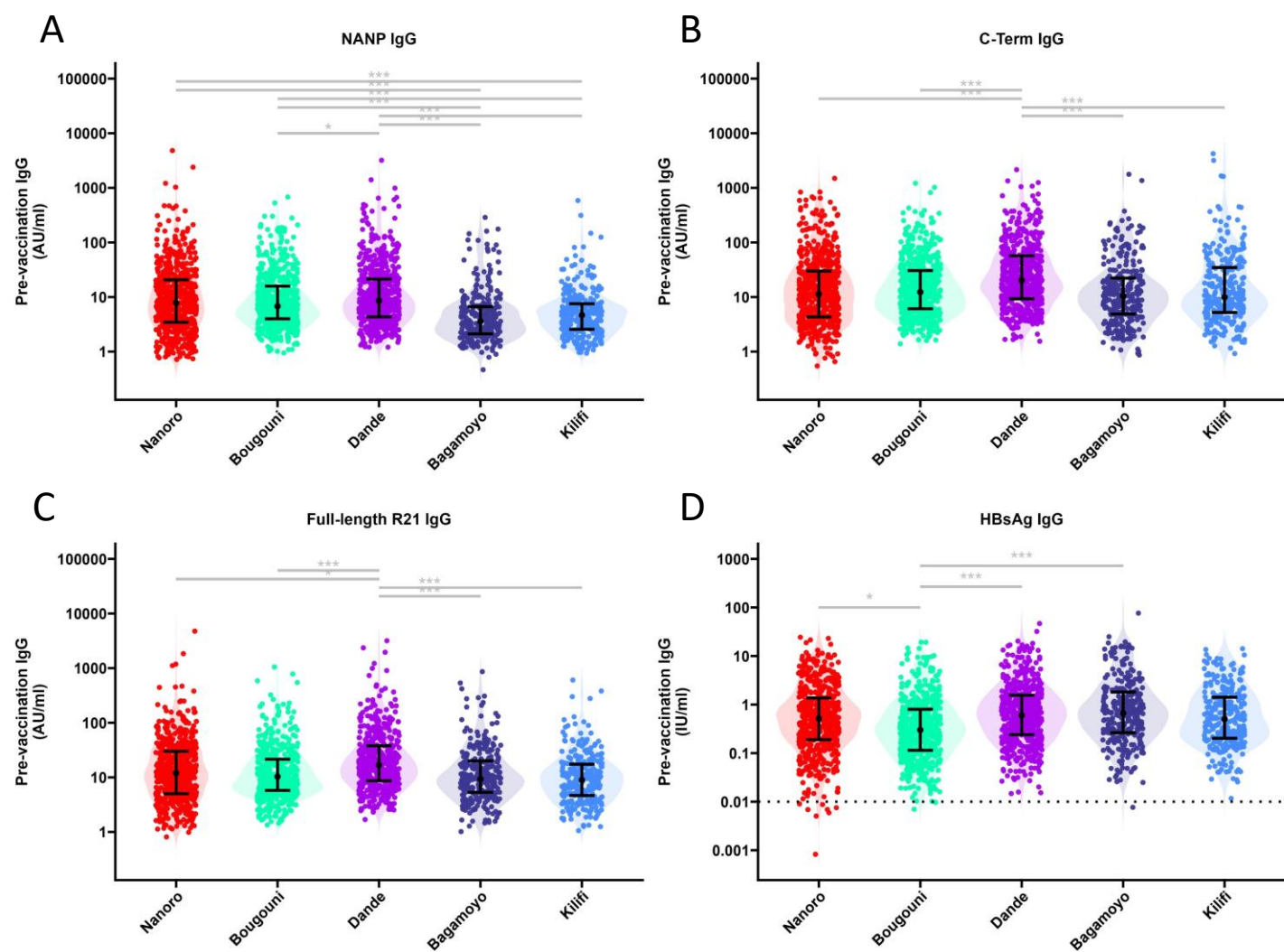


Figure S2 – Pre-vaccination IgG levels by site.

Levels of IgG antibody to A. NANP, B C-term, C. full-length R21 vaccine and D. hepatitis B surface antigen (HBsAg) were measured in pre-vaccination (PV) samples. Asterisks represent results of post-hoc pairwise Dunn's tests between sites with Bonferroni correction. (*) $p < 0.05$, (***) $p < 0.001$.

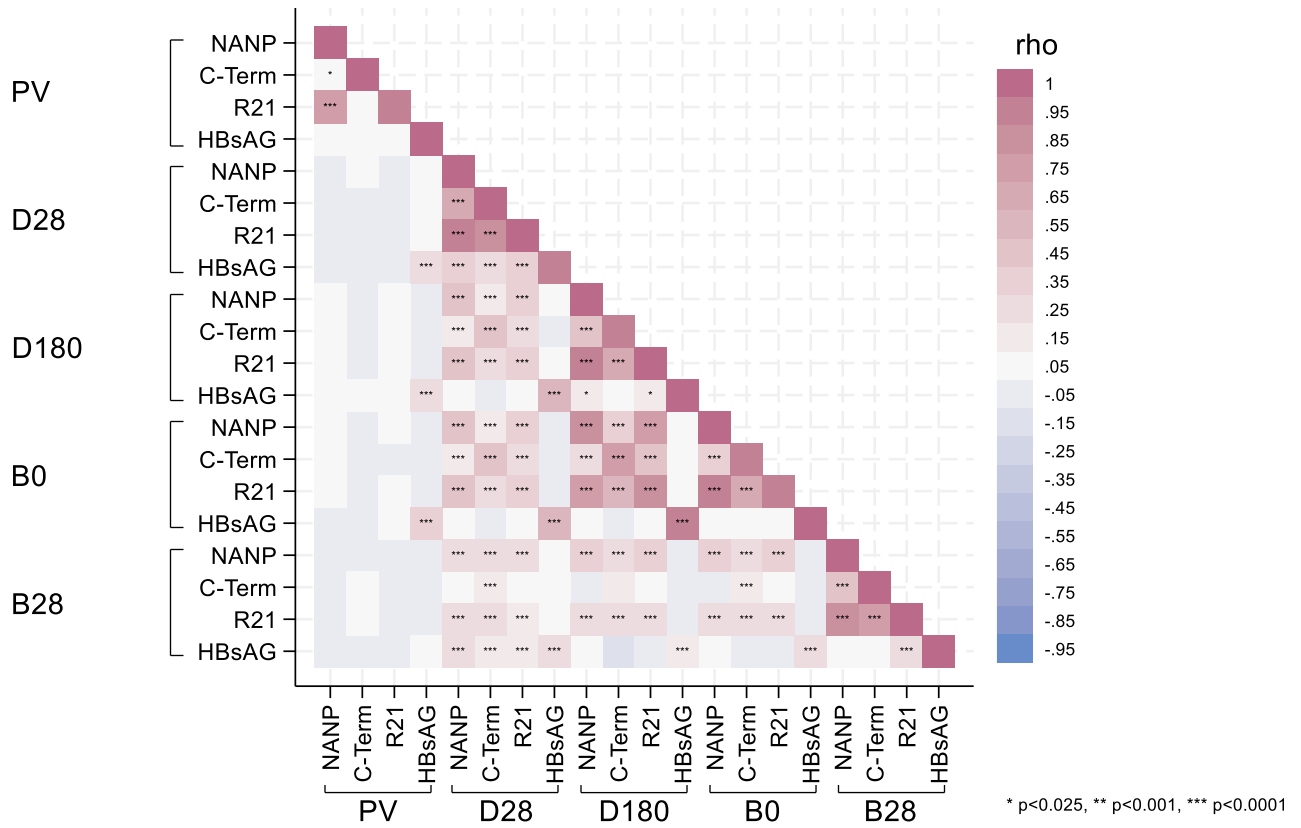


Fig S3 – Heatplot of all antibody levels at all timepoints for R21/Matrix-M™ vaccinated individuals only. All sites and age groups combined.

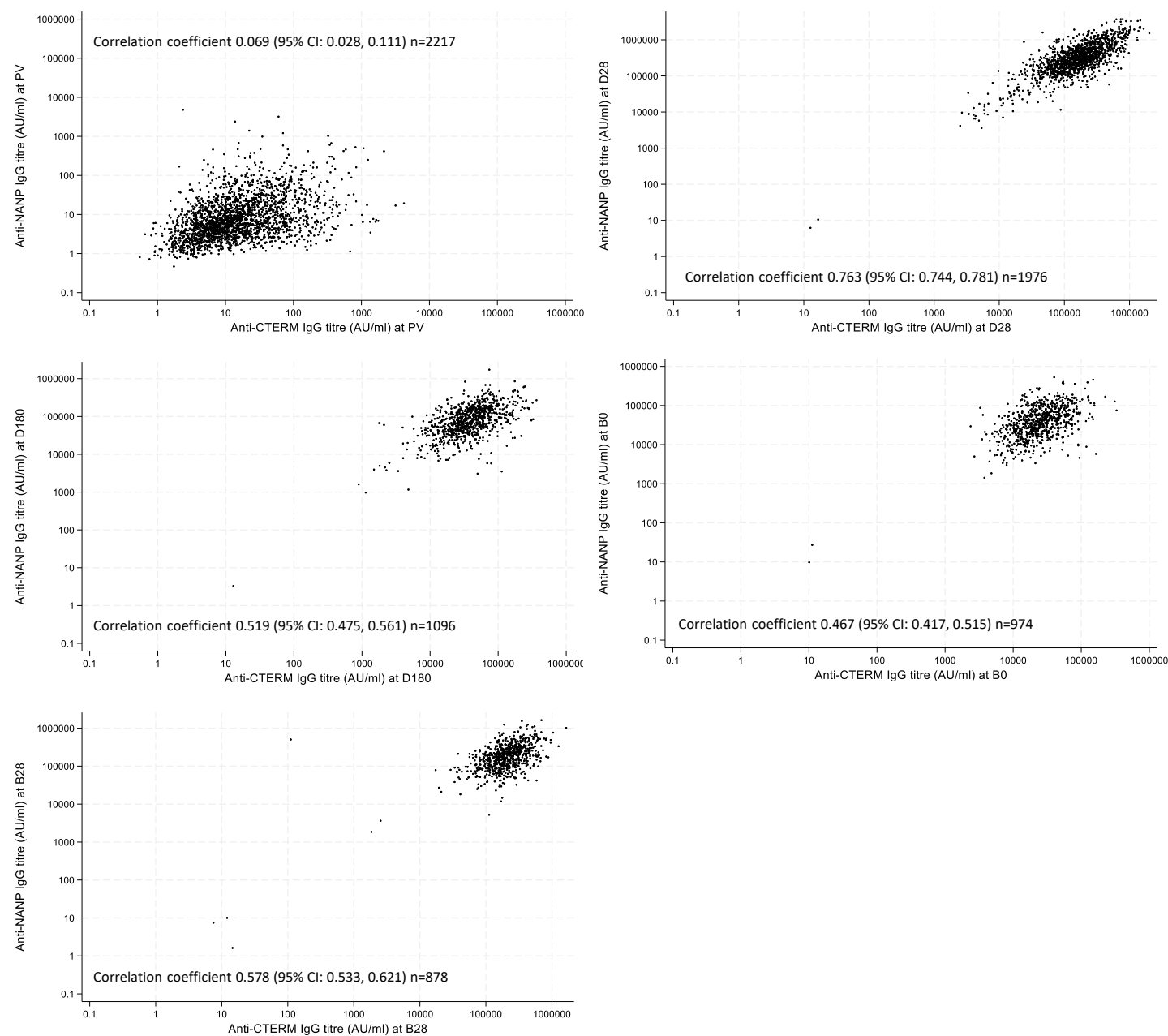


Figure S4 Correlation between anti-NANP IgG titres and anti-C-Term IgG titres before first dose (PV), 28 (D28), 180 (D180) and 360 (B0) days after third dose and 28 days (B28) after first booster.
PV plot includes participants from both control and R21/Matrix-M™ study arms and all other plots only include the R21/Matrix-M™ study arm.

	N	Rate (events / PYR)	IRR* (95% CI)	p-value
D28				
NANP	1369	0.19 (244/1290)	0.63 (0.44, 0.90)	0.0109
CTERM	1369	0.19 (243/1290)	0.64 (0.45, 0.91)	0.0126
R21	1288	0.20 (239/1214)	0.68 (0.48, 0.95)	0.0250
HBsAg	1343	0.19 (240/1266)	0.84 (0.64, 1.09)	0.1969
D180				
NANP	884	0.20 (167/840)	0.56 (0.34, 0.94)	0.0272
CTERM	865	0.20 (166/822)	0.41 (0.23, 0.70)	0.0014
R21	860	0.20 (166/817)	0.48 (0.27, 0.84)	0.0100
HBsAg	899	0.20 (168/854)	0.83 (0.57, 1.20)	0.3100
B0				
NANP	827	0.20 (154/786)	0.68 (0.40, 1.17)	0.1627
CTERM	775	0.20 (150/737)	0.32 (0.17, 0.61)	0.0005
R21	772	0.20 (148/735)	0.48 (0.25, 0.92)	0.0281
HBsAg	824	0.20 (154/783)	0.82 (0.55, 1.24)	0.3479

Table S6 - Change in rate of malaria episodes by antibody level.

Incidence Rate Ratio of clinical malaria per 10 fold increase in the IgG antibody titre in the R21/Matrix-M™ study arm measured at 28 (D28), 180 (D180) and 365 (B0) days after the third vaccine dose for four antigens during the first 12 months following the primary series. IRR - incidence rate ratio, PYR - person-years at risk, *adjusted for site

Study site	VE* (95% CI)	NANP Threshold Correlate	CTERM Threshold Correlate
All	72.27 (68.79, 75.35)	184887 (170093, 202856)	98458 (90259, 109279)
Nanoro	72.10 (67.24, 76.24)	176038 (162868, 196458)	89241 (78709, 97429)
Bougouni	81.81 (75.34, 86.59)	186751 (159605, 232253)	89024 (69416, 110384)
Dande	72.80 (64.05, 79.41)	161476 (128628, 198693)	98651 (82328, 121807)
Bagamoyo	53.31 (24.15, 71.26)	346441 (227869, 818763)	212058 (144131, 415480)
Kilifi	57.75 (33.72, 73.07)	195792 (91480, 398650)	93612 (42859, 188458)

Table S7 - Threshold correlate for NANP and C-Term IgG in AU/ml based on the D28 (1 month post 3rd dose)

*Vaccine efficacy calculated as 1-hazard ratio from a Cox regression model including all trial participants in the modified per-protocol population, adjusting for age at randomisation, sex, bed net use and number of SMC rounds

SMC Rounds*	Nanoro n(%)	Bougouni n(%)	Dande n(%)	Bagamoyo n(%)	Kilifi n(%)
R21/Matrix-M™					
0	17 (4.3%)	19 (5.0%)	0 (0.0%)	194 (100.0%)	197 (100.0%)
1	34 (8.6%)	181 (47.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
2	72 (18.2%)	94 (24.7%)	1 (0.3%)	0 (0.0%)	0 (0.0%)
3	91 (23.0%)	70 (18.4%)	21 (5.6%)	0 (0.0%)	0 (0.0%)
4	182 (46.0%)	17 (4.5%)	356 (94.2%)	0 (0.0%)	0 (0.0%)
Control					
0	8 (4.1%)	13 (6.9%)	0 (0.0%)	95 (100.0%)	95 (100.0%)
1	16 (8.1%)	83 (43.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
2	34 (17.3%)	45 (23.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
3	54 (27.4%)	40 (21.1%)	10 (5.4%)	0 (0.0%)	0 (0.0%)
4	85 (43.2%)	8 (4.1%)	176 (94.6%)	0 (0.0%)	0 (0.0%)

Table S8 Number of SMC rounds given by site and study arm.

Among immunogenicity cohort only. SMC was given as part of routine health care. East African sites (Bagamoyo and Kilifi) do not routinely administer SMC. *given in 2021.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [R21MatrixMTrialGroupAuthorsFeb2026.pdf](#)
- [RSStockdale.pdf](#)