



Duration and association with protection of NANP-repeat-specific and C-terminus-specific anti-circumsporozoite protein IgG responses following RTS,S/AS01_E vaccination: an observational ancillary immunological study of a phase 3 clinical trial

Maria Lara-Escandell*, Lina Sánchez*, Dídac Macià, Chenjerai Jairoce, Maxmillian Mpina, Hermann Sorgho, Selidji Todagbe Agnandji, David Dosoo, Ben Gyan, Marta Vidal, Alfons Jiménez, Robert Andrew Mitchell, Daniel Medina-Expósito, Laura Puyol, Rebeca Santano, Sheetij Dutta, Seth Owusu-Agyei, Kwaku Poku Asante, Halidou Tinto, Pedro Aide, Augusto Nhabomba, Benjamin Mordmüller, Claudia Daubenberger, Ruth Aguilar, John Waitumbi, Gemma Moncunill†, Carlota Dobaño†



Summary

Background The RTS,S/AS01_E malaria vaccine provides partial protection against *Plasmodium falciparum*, largely mediated by antibodies targeting the circumsporozoite protein. Correlates of protection remain incompletely defined and have focused mainly on peak IgG responses to the immunodominant NANP-repeats, with less known about antibody durability or responses to the C-terminus of circumsporozoite protein.

Methods In this observational study, we evaluated IgG responses to NANP-repeat, C-terminus, and full-length circumsporozoite protein constructs at five timepoints before and after primary and booster vaccination, in children aged 5–17 months from six African countries enrolled in the RTS,S/AS01_E phase 3 trial (NCT00866619, 2009–11). Antibody kinetics were analysed with linear regressions, and associations with clinical malaria risk over 1 year with Cox models.

Findings Between May 25, 2009, to March 5, 2010, 1292 children were recruited and included for analysis in this study. IgG levels declined more slowly for C-terminus than for NANP-repeat over 17 months after primary vaccination (geometric mean fold change 0.099 [95% CI 0.089–0.110] vs 0.055 [0.048–0.062]; $p < 0.0001$). The booster significantly enhanced C-terminal IgG, exceeding post-primary peak levels (geometric mean fold change 1.98 [95% CI 1.77–2.21]), whereas NANP-repeat responses were restored but remained below post-primary levels (geometric mean fold changes 0.57 [95% CI 0.51–0.63]). Higher circumsporozoite protein IgG levels correlated with reduced malaria risk, particularly during maintenance phases, with stronger associations for C-terminus, which remained independently protective after adjustment for NANP-repeat antibodies (hazard ratio 0.60, 95% CI 0.43–0.83; $p = 0.0021$). After boosting, maintenance but not peak IgG levels correlated with reduced malaria risk.

Interpretation IgG to circumsporozoite protein C-terminus are more durable than those to the NANP-repeats. Antibody maintenance emerges as a stronger predictor of RTS,S/AS01_E vaccine efficacy than peak responses.

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Introduction

Malaria is a major global health challenge, with an estimated 282 million cases and 610 000 deaths in 2024, most of which were in sub-Saharan African children younger than 5 years.¹ RTS,S/AS01_E (RTS,S) is a subunit vaccine targeting the *Plasmodium falciparum* circumsporozoite protein, incorporating 18 NANP repeats and the adjacent C-terminal region. The circumsporozoite protein fragment is fused to the

hepatitis B surface antigen (HBsAg) and co-formulated with the AS01 adjuvant.² RTS,S and R21/Matrix-M (R21), which are based on the same immunogen, are the only WHO-approved malaria vaccines that have been integrated into immunisation programmes in children aged 5 months and older who live in moderate-to-high malaria transmission areas of Africa.^{3–5}

Although RTS,S represents a major advance in malaria control, its efficacy is modest and short-lived.² In a large

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*Joint first authors

†Joint last authors

ISGlobal, Barcelona, Spain (M Lara-Escandell MSc, L Sánchez MSc, D Macià PhD, M Vidal MSc, A Jiménez MSc, R A Mitchell PhD, D Medina-Expósito, L Puyol MSc, R Santano PhD, R Aguilar PhD, G Moncunill PhD, Prof C Dobaño PhD); Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Spain (M Lara-Escandell, L Sánchez, R A Mitchell); Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Barcelona, Spain (D Macià, R Santano, Prof B Mordmüller PhD, G Moncunill, Prof C Dobaño); Manhica Health Research Center (CISM), Maputo, Mozambique (C Jairoce PhD, P Aide PhD, A Nhabomba PhD); Biomedical Research and Clinical Trials Department, Ifakara Health Institute, Tanzania (M Mpina PhD); Unité de Recherche Clinique de Nanoro, Institut de Recherche en Sciences de la Santé, Nanoro, Burkina Faso (H Sorgho PhD, Prof H Tinto PhD); Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon (S T Agnandji PhD); Institute of Medical Microbiology, University Hospital Münster, Münster, Germany (S T Agnandji); Kintampo

Health Research Centre,
Research & Development
Division, Ghana Health Service,
Kintampo, Ghana

(D Dosoo PhD, B Gyan PhD,
Prof S Owusu-Agyei PhD,
K P Asante, PhD); **Noguchi
Memorial Institute for Medical
Research, University of Ghana,
Accra, Ghana** (B Gyan); **Centro
de Investigación Biomédica en
Red de Epidemiología y Salud
Pública (CIBERESP), Barcelona,
Spain** (A Jiménez); **Walter Reed
Army Institute of Research,
Silver Spring, MD, USA**
(S Dutta PhD); **Department of
Medical Microbiology,
Radboud University Medical
Center, Nijmegen, Netherlands**
(Prof B Mordmüller); **University
of Basel, Basel, Switzerland**
(Prof C Daubenberger PhD);
**Swiss Tropical and Public
Health Institute, Allschwil,
Switzerland**
(Prof C Daubenberger); **US Army
Medical Research Directorate-
Africa, Kenya Medical Research
Institute, Nairobi, Kenya**
(J Waitumbi PhD)

Correspondence to:
Dr Gemma Moncunill, ISGlobal,
08036, Barcelona, Spain
gemma.moncunill@isglobal.
org

or
Prof Carlota Dobaño, ISGlobal,
08036, Barcelona, Spain
carlota.dobano@isglobal.org

Research in context

Evidence before this study

RTS,S/AS01_E (RTS,S) and R21/Matrix-M are the first malaria vaccines approved by WHO for use in children from 5 months of age living in sub-Saharan African areas of moderate-to-high intensity of *Plasmodium falciparum* transmission. We searched PubMed for studies published between database inception and Dec 29, 2025, using the terms [“RTS,S” OR “malaria vaccine”] AND [“antibodies” OR “immunogenicity” OR “correlates of protection”]. We found that IgG antibody responses, specifically against the NANP repetitions of the circumsporozoite protein present in the RTS,S vaccine, was the primary correlate of protection that has been suggested thus far. This finding was primarily established through immunogenicity and efficacy analyses derived from four field studies conducted across 11 African countries as part of the paediatric RTS,S phase 3 trial; pooled analyses of five phase 2 trials in infants and children from Mozambique, Tanzania, and Kenya; and four phase 2 trials in malaria-naïve adult populations.

Vaccine-induced immune response studies have primarily focused on IgG responses to the NANP-repeat, known to be the immunodominant B-cell epitope of circumsporozoite protein, which correlate with vaccine efficacy. Monoclonal antibodies targeting this region have also been shown to mediate protection through passive immunisation. By contrast, the circumsporozoite protein C-terminal domain is less studied, despite containing multiple T-cell epitopes and eliciting strong antibody responses. Anti-C-terminal IgG has been associated with RTS,S-mediated protection in phase 2 and 3 trials and controlled human malaria infection studies, although extensive detailed characterisation of C-terminal-specific responses remains insufficient. Furthermore, the short-lived protection conferred by RTS,S has been associated with the rapid decay of anti-NANP-repeat IgG titres following immunisation, with data from the phase 3 clinical trial.

Added value of this study

We used multiplex quantitative suspension array technology to measure IgG levels to circumsporozoite protein full-length, NANP repeat, and C-terminus in 1292 African children from the

RTS,S phase 3 trial in six settings of different malaria endemicity. Unlike other immunogenicity studies focusing only on NANP repeat peak responses, we also evaluated long-term antibody maintenance for each distinct circumsporozoite protein construct. Furthermore, this study is the first to investigate the differential kinetics of the IgG response between NANP repeat and C-terminus through five study visits with a large sample size, and to associate these responses with clinical malaria risk after primary and booster vaccination over time.

This approach shows that antibody durability rather than peak magnitude is a stronger correlate of protection, and provides evidence that C-terminal-specific IgGs are longer-lived than NANP-specific IgG and may be independently associated with reduced malaria risk after primary vaccination. By demonstrating preferential boosting and enhanced persistence of C-terminal responses, this study refines current correlates of protection and offers actionable guidance for the design of next-generation circumsporozoite protein-based malaria vaccines, which should prioritise sustained and broader humoral immunity over short-lived peak responses.

Implications of all the available evidence

Understanding the longitudinal kinetics of RTS,S-induced antibodies and knowing the epitope specificities over time can guide public health policy makers in developing better vaccine strategies and schedules to improve vaccine efficacy. The evidence supports shifting the focus in malaria vaccine evaluation and design towards immune maintenance, and booster strategies that preferentially enhance long-lived responses. For second-generation circumsporozoite protein vaccines, our results argue (1) for the retention and optimisation of C-terminal epitopes that seem to independently mediate protection, (2) the incorporation of platforms or adjuvants that promote durable humoral immunity, and (3) the use of antibody maintenance rather than peak response as a key immunological endpoint in clinical trials. This paradigm might accelerate the development of vaccines with higher and longer-lasting efficacy, advancing global malaria control goals.

phase 3 trial conducted across seven African countries, the RTS,S malaria vaccine, which was administered as a three-dose primary series in monthly intervals, had a vaccine efficacy of 55·8% during the 12 months after completion of the three-dose primary series in children aged 5–17 months. Vaccine efficacy decreased to 45·1% over 18 months and 35·2% over 32 months. A booster dose given 18 months after the primary series partly restored protection, increasing vaccine efficacy to 43·9% during the 32 months.²

Immunogenicity studies have largely focused on the IgG response to the NANP repeat, which is the circumsporozoite protein immunodominant B-cell epitope^{6,7}, after primary vaccination.^{2,8,9} However, the

C-terminal domain contains multiple T-cell epitopes within the Th2R and Th3R regions^{10,11} and also induces antibody responses.^{9,12–14} NANP-specific antibodies have been correlated with vaccine efficacy^{2,12–14} and human monoclonal antibodies can be used for passive immunisation.¹⁵ By contrast, data on C-terminal responses are more sparse, although some studies, including our previous work, have shown that C-terminal IgGs are linked to RTS,S-mediated protection.^{12,14,16,17}

The limited duration of protection conferred by RTS,S has been linked to a rapid decay in anti-NANP IgG titres following the primary immunisation schedule.⁸ We hypothesised that IgG responses to the C-terminus and NANP epitopes might be different in their magnitude

and durability following primary and booster RTS,S vaccination, and their association with protection might be independent from one another. In this study, we investigated the immunogenicity and kinetics of IgG antibodies to NANP and C-terminal regions as induced by three or four RTS,S doses in a large set of children from a phase 3 trial across six African sites of different malaria transmission intensities. We also examined whether peak response and persistence of these IgG levels were linked to protection against clinical malaria and evaluated their potential as correlates of protection.

Methods

Study design and population

This observational study comprised a subset of children aged 5–17 months from the MAL067 study ancillary to the phase 3 randomised clinical trial MAL055 (NCT00866619; appendix 1 pp 4, 6), based in seven countries in sub-Saharan Africa. Recruitment for this study took place between May 25, 2009, and March 5, 2010. The MAL055 study was conducted in 11 health centres² and involved collaboration with research and medical institutions.

Participating sites differed in malaria transmission intensities and were classified depending on the malaria annual incidence at the time of the trial, as low (0·5 or lower *Plasmodium falciparum* episodes per person-year) or high (above 0·5). Incidence was measured in the MAL055 trial infant comparator arm during the first year of follow-up.¹⁸ Low malaria transmission intensity sites consisted of Manhiça, Mozambique (0·13 episodes per person-year), Lambaréné, Gabon (0·19 episodes per person-year), and Bagamoyo, Tanzania (0·21 episodes per person-year). High malaria transmission intensity sites consisted of Kombewa, Kenya (2·23 episodes per person-year; perennial transmission), Kintampo, Ghana (2·29 episodes per person-year; seasonal transmission), and Nanoro, Burkina Faso (3·32 episodes per person-year; seasonal transmission).

The study protocol of the immunology ancillary study MAL067 was approved by the Ethics Committees from Spain (Comité Ético d'Investigació Clínica, Hospital Clínic Research Ethics Committee, Barcelona, 2008/4622), Mozambique (Comité Nacional de Bioética para Saúde, Manhiça, 332/CNBS/18), Tanzania (The Ifakara Health Institute Review Board, 14-2017), Kenya (The Kenya Medical Research Institute Scientific and Ethics Review Unit, 1461B), Burkina Faso (Comité d'Etique pour la Recherche en Santé, number 2010-15), Comité d'Etique Centre Muraz, 001-2010/CE-CM), Gabon (Comité d'Etique Régional Indépendant de Lambaréné, 110021), and Ghana (Noguchi Memorial Institute for Medical Research Institutional Review Board, 048/08-09; Kintampo Health Research Centre Institutional Ethics Committee, 2009-4).

Participants from the ancillary MAL067 study were selected based on sample availability, prioritising

children with the greatest number of plasma or serum and peripheral blood mononuclear cells samples across five timepoints (appendix 1 pp 2, 5). Following information activities in community meetings and a positive recommendation from community leaders, the parents or guardians of children in the eligible age groups were approached. Written informed consent was obtained from the parents or guardians of all participants before recruitment.

Procedures

In the MAL055 trial, children were assigned to each study group using block randomisation to receive either three doses of RTS,S plus an RTS,S booster at month 20 (R3R), three doses of RTS,S plus a comparator booster (R3C), or three comparator doses plus a comparator booster (C3C). Doses were administered by local investigator teams who also collected serum or plasma samples were collected at five visits: baseline (month 0), 1 month after primary vaccination (month 3), 18 months after the primary series (month 20), 1 month after the booster (month 21), and 1 year after the booster (month 32). Data on participant sex were collected from parents or guardians at the time of enrolment into the MAL055 phase 3 trial.

IgG levels to three recombinant circumsporozoite protein constructs (full-length,¹⁹ C-terminus,²⁰ and NANP)¹⁴ were quantified by suspension array technology (x-MAP Luminex; appendix 1 pp 2–3). IgG levels were calibrated to arbitrary units (AU) to ensure linearity across serial dilutions, using an internal positive control curve included in each plate. Furthermore, the internal positive control was titrated against the external WHO *P. falciparum* reference pool (NIBSC 10/198). For each circumsporozoite protein construct, 100 AU were defined as equivalent to the IgG levels in the WHO reference pool.

Outcomes

For the immunogenicity analysis, the primary outcome was antibody kinetics, including boosting and waning, calculated as a ratio of geometric mean IgG levels between two specific timepoints: month 21/20, month 21/3, month 20/3, and month 32/21. Secondary outcomes included the assessment of factors associated with IgG responses and kinetics, such as age, sex, study site, haemoglobin level, anaemia status, height-for-age Z score (HAZ-score) at baseline, weight-for-age Z score (WAZ-score) at baseline, malaria transmission intensity, parasitaemia at month 20, number of malaria episodes during the first 12 months of follow-up, and distance of the child's home to the outpatient health facility. We also assessed the correlation between IgG levels for C-terminus and NANP at each study timepoint.

For the protection analysis, clinical malaria cases were defined as fever (axillary temperature $\geq 37\cdot5^{\circ}\text{C}$) or history of fever within 24 h and asexual *P. falciparum* parasitaemia

See Online for appendix 1

by microscopy (>0 parasites per μL). The primary outcome was the association between circumsporozoite protein IgG levels at the peak or maintenance phases and the hazard of clinical malaria over 1 year of follow-up after primary series or booster vaccinations. Secondary analyses included assessment of independent associations, through models adjusting for IgG levels binding the alternative circumsporozoite protein construct, and vaccine efficacy–IgG curve estimations through the inclusion of the comparator arm in the models.

Statistical analysis

Sample size was determined based on practical considerations, with the aim of exceeding that of other previous analyses that were powered to assess immunogenicity.

IgG levels were $\log_{10}$ -transformed before analysis. IgG geometric means were compared between timepoints using a linear mixed-effects model with random intercept for each participant, adjusted for site, sex and continuous age, and with inference based on t tests using the Satterthwaite approximation for degrees of freedom.²¹ IgG geometric mean fold changes were obtained through exponentiation of fixed effects regression coefficients. To compare IgG geometric mean fold changes between circumsporozoite protein constructs, we used a model with an interaction between timepoint and construct.

For the analysis of determinants of immunogenicity, IgG levels stratified by malaria transmission intensities were compared using unpaired t tests and linear regression models adjusted by site, which were fitted separately for age, sex, HAZ-score, WAZ-score, and haemoglobin. IgG levels at month 21 were compared between subjects with and without parasitaemia at month 20 using linear regression models adjusted by site and age.

For the correlates of protection analyses, site-stratified Cox regression models with robust variance estimation were used to account for within-individual correlation across multiple episodes, adjusting for sex and continuous age. Effect sizes were reported as hazard change ratio per ten-fold IgG increase (HR) per ten-fold increase in IgG AU. Models were extended to include comparator recipients and IgG vaccination group interactions to estimate vaccine efficacy across the observed range of IgG values. The relationship of log-hazard and log-IgG levels was modelled linearly and using natural cubic splines; knot placement was defined by quantiles, and the number of knots was selected by minimising the Akaike Information Criterion (AIC). As complementary analyses, models were further adjusted for responses to other circumsporozoite protein constructs to assess independent associations. In these cases, the adjusting IgG covariates were modelled with natural cubic splines. Statistical significance was set at $p < 0.05$. p values from analyses involving multiple

tests were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. Results derived from peak or maintenance IgG levels were not directly comparable because sample sizes and individuals included in each analysis differed slightly due to sample availability; we therefore performed a sensitivity analysis restricted to participants with complete data (ie, complete-case analysis) to assess the robustness of our findings.

All statistical analyses were conducted in R (version 4.5.1), using the survival package²² for time-to-event analyses, which implements stratified Andersen–Gill Cox regression and weighted Nelson–Aalen cumulative hazard estimation, and lme4 (lmer) with lmerTest²¹ for linear mixed-effects modelling.

Role of the funding source

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

Results

Between May 25, 2009, and March 5, 2010, 3630 children aged 5–17 months were enrolled in the MAL067 study ancillary to the phase 3 randomised clinical trial MAL055. Of this group, 1292 (35.6%) were included in this study for analysis (R3R $n=411$, R3C $n=446$, C3C $n=435$; table).

Peak anti-circumsporozoite protein IgG responses measured 1 month after primary RTS,S vaccination (month 3) waned by month 20 at a significantly faster rate ($p < 0.0001$ for the interaction) for the NANP repeat (geometric mean fold change 0.055 [95% CI 0.048–0.062]) than for the C-terminal antibodies (0.099 [0.089–0.110]; figure 1; appendix 1 p 8). The decline of IgG to circumsporozoite protein full-length was between that to NANP and C-terminus (appendix 1 p 7). Following a booster dose in the R3R group, anti-NANP IgG levels at month 21 increased but remained lower than those at month 3 (0.57 [0.51–0.63]), whereas C-terminal-specific IgG levels significantly surpassed month 3 values (1.98 [1.77–2.21]; figure 1A, B). The waning of IgG levels after booster vaccination was again more pronounced for NANP (0.118 [0.106–0.13]) than for C-terminus (0.21 [95% CI 0.19–0.23]; $p < 0.0001$; figure 1C), indicating a greater durability of C-terminal-specific antibodies. Although these differences in IgG geometric mean fold changes were statistically significant given the large sample size, the corresponding effect sizes were small when compared with the substantial heterogeneity in IgG levels across participants at any given timepoint (figure 1A) and in individual-level kinetic changes (figure 1B, C).

At the individual level, IgG responses to NANP and C-terminus moderately correlated at months 3, 20, 21, and 32 in the R3R group (Pearson's $r=0.69$, 0.67, 0.64, and 0.53, respectively), indicating that individuals with

high antibody levels to one circumsporozoite protein epitope also tended to have relatively high levels to the other (appendix 1 p 9). Of note, this correlation decreased at later timepoints after waning (months 20 and 32), probably suggesting different factors affecting the decay of C-terminus compared with NANP-specific antibodies.

After administering primary and booster RTS,S doses, there was no significant difference in circumsporozoite protein IgG levels between high and low malaria transmission sites at months 3 and 21, indicating no effect modification by malaria transmission intensity on vaccine immunogenicity and an equaliser effect, despite pre-vaccination anti-circumsporozoite protein IgG level differences (appendix 1 pp 10–12).

Regarding other factors affecting RTS,S immunogenicity, circumsporozoite protein IgG levels at month 3 significantly decreased with age (geometric mean fold changes per week of age: NANP 0.993 [95% CI 0.989–0.997]; C-terminus 0.993 [0.990–0.997]; full length 0.993 [0.990–0.997]; appendix 1 pp 13–14). No association was found at months 20, 21, or 32 (appendix 1 p 15). Finally, IgG levels against C-terminus and full-length circumsporozoite protein were significantly higher in individuals with patent parasitaemia at month 20 compared with those without parasitaemia ($p=0.050$ and $p=0.015$, respectively), whereas no significant difference was observed for NANP-specific IgG responses ($p=0.15$; appendix 1 p 16).

Following primary vaccination, high-RTS,S responders (IgG levels above the median) at month 3 had a significantly lower site-adjusted cumulative clinical malaria hazard than low-RTS,S responders, and both showed lower cumulative hazard curves than the comparator group (figure 2A). In Cox proportional hazard models, this finding was also observed for both NANP (HR 0.69 [95% CI 0.55–0.87]; $p=0.0014$) and for C-terminus (0.68 [0.51–0.90]; $p=0.0082$; figure 2A, E). However, after adjustment for the responses to the other circumsporozoite protein construct, neither NANP nor C-terminus retained an independent association with reduced risk (figure 2E).

The same analysis was done using IgG levels measured 18 months after primary vaccination (ie, month 20) as an indicator of antibody maintenance (figure 2C). For NANP-specific IgG levels, similar responses were obtained compared with peak responses (HR 0.62 [95% CI 0.51–0.75]; $p<0.0001$). However, for C-terminal-specific IgG levels, the protective association appeared to be stronger for levels 18 months after primary vaccination rather than immediately after vaccination (0.51 [0.39–0.66]; $p<0.0001$), and remained significant even after adjusting for NANP IgG levels (0.60 [0.43–0.83]; $p=0.0021$; figure 2E).

One month after booster (ie, month 21), R3R recipients also had a lower cumulative clinical malaria hazard than comparators and site-adjusted cumulative hazard curves

	R3R (n=411)	R3C (n=446)	C3C (n=435)
Sex			
Female	201 (48.9%)	243 (54.5%)	236 (54.3%)
Male	210 (51.1%)	203 (45.5%)	199 (45.7%)
Malaria transmission intensity			
Low malaria transmission intensity	213 (51.8%)	243 (54.5%)	238 (54.7%)
High malaria transmission intensity	198 (48.2%)	203 (45.5%)	197 (45.3%)
Site			
Manhiça, Mozambique	85 (20.7%)	91 (20.4%)	103 (23.7%)
Lambaréné, Gabon	41 (10.0%)	53 (11.9%)	50 (11.5%)
Bagamoyo, Tanzania	87 (21.2%)	99 (22.2%)	85 (19.5%)
Kombewa, Kenya	89 (21.7%)	88 (19.7%)	93 (21.4%)
Kintampo, Ghana	45 (10.9%)	51 (11.4%)	38 (8.7%)
Nanoro, Burkina Faso	64 (15.6%)	64 (14.3%)	66 (15.2%)
Haemoglobin (g/dL)			
Mean (SD)	9.63 (1.39)	9.59 (1.45)	9.54 (1.55)
Median (min to max)	9.7 (5.5 to 14.8)	9.7 (5.2 to 14.7)	9.7 (5.0 to 17.9)
Missing	1 (0.2%)	0	0
Height-for-age Z-score			
Mean (SD)	-1.04 (1.28)	-1.01 (1.27)	-1.12 (1.30)
Median (min to max)	-1.04 (-5.85 to 2.92)	-0.99 (-5.23 to 4.73)	-1.10 (-5.72 to 3.57)
Missing	0	1 (0.2%)	0
Weight-for-age Z-score			
Mean (SD)	-0.83 (1.24)	-0.82 (1.19)	-0.84 (1.24)
Median (min to max)	-0.77 (-4.34 to 2.48)	-0.80 (-4.61 to 2.28)	-0.77 (-4.36 to 3.19)
Anaemia (<8 g/dL)			
No	362 (88.1%)	391 (87.7%)	369 (84.8%)
Yes	48 (11.7%)	55 (12.3%)	66 (15.2%)
Missing	1 (0.2%)	0 (0%)	0
Distance to outpatient facility (km)			
Mean (SD)	3.76 (4.39)	4.20 (5.40)	3.39 (4.38)
Median (min to max)	2.0 (0.10 to 30.0)	2.2 (0.20 to 50.0)	2.0 (0.10 to 30.0)
Clinical malaria cases in the first 12 months			
None	238 (57.9%)	279 (62.6%)	224 (51.5%)
1	68 (16.5%)	76 (17.0%)	51 (11.7%)
2+	105 (25.5%)	91 (20.4%)	160 (36.8%)

R3R=three doses of RTS,S/AS01, and a RTS,S/AS01, booster at month 20. R3C=three doses of RTS,S/AS01E and a comparator booster. C3C=three doses of a comparator vaccine and a comparator booster. Areas of low malaria transmission intensity: Bagamoyo, Lambaréné, and Manhiça; areas of high malaria transmission intensity: Kintampo, Kombewa, and Nanoro.

Table: Demographic and clinical characteristics of the study population

largely overlapped for high and low responders (figure 2B). Likewise, in proportional hazard models, neither NANP-specific nor C-terminal-specific IgG levels were associated with a significant hazard reduction (NANP: HR 0.91 [95% CI 0.61–1.36]; $p=0.64$; C-terminus: 0.97 [0.59–1.62]; $p=0.92$; figure 2F). By contrast, IgG levels measured 12 months after the booster (ie, month 32), as an indicator of maintenance, were associated with decreased malaria risk (NANP: 0.66 [0.50–0.86]; $p=0.0021$; C-terminus: 0.55 [0.34–0.88]; $p=0.013$). In models adjusting for the other CSP

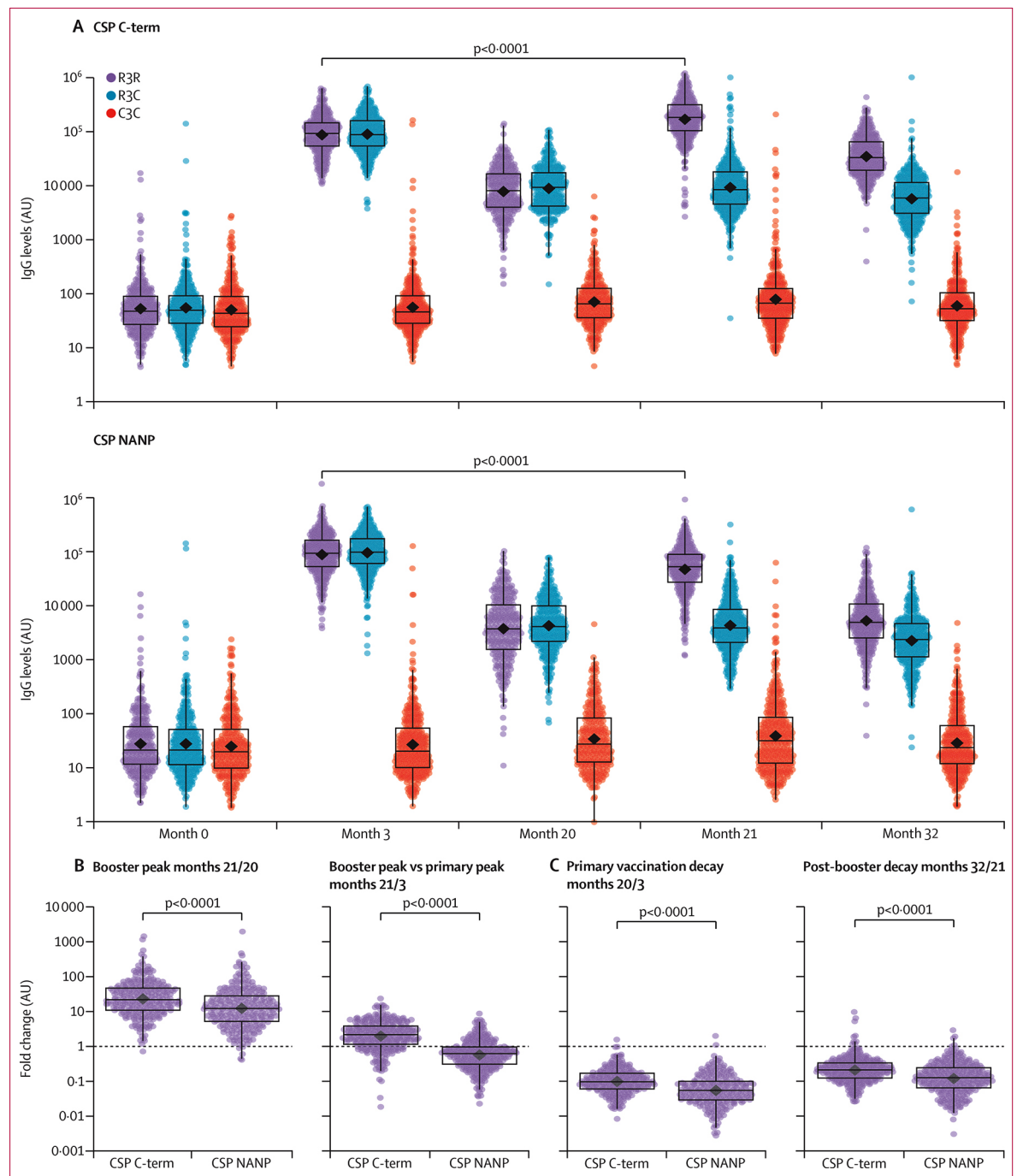


Figure 1: IgG levels against NANP and C-terminal circumsporozoite protein constructs before and after vaccination doses.

(A) IgG levels ($\log_{10}$ AU) for each vaccination group over time. Statistical significance of differences in IgG levels between visits at months 3 and 21 assessed with a linear mixed-effects model adjusted by site, age and sex. (B) Fold changes in IgG levels between months 21/20 and months 21/3, were compared for NANP and CSP C-term. Individual fold-changes were calculated between two visits as $10^{(\log_{10}(\text{AU}_{TP2}) - \log_{10}(\text{AU}_{TP1}))}$ only in individuals who had available samples for both timepoints. (C) Fold changes in IgG levels between months 20/3 and 32/21, were analysed for NANP and CSP C-term responses. p-values were calculated using a model with an interaction between timepoint and construct. All boxplots indicate geometric mean (diamond), median (IQR), upper and lower whiskers correspond to the $Q_3 + 1.5 \times \text{IQR}$ and $Q_1 - 1.5 \times \text{IQR}$, respectively. Vaccination groups: R3R (purple), received three doses of RTS,S/AS01_E followed by an RTS,S/AS01_E booster; R3C (blue), RTS,S/AS01_E followed by a comparator booster; and C3C (orange) received four comparator doses. AU=arbitrary units. CSP=circumsporozoite protein. C-term=C-terminal. TP=timepoint.

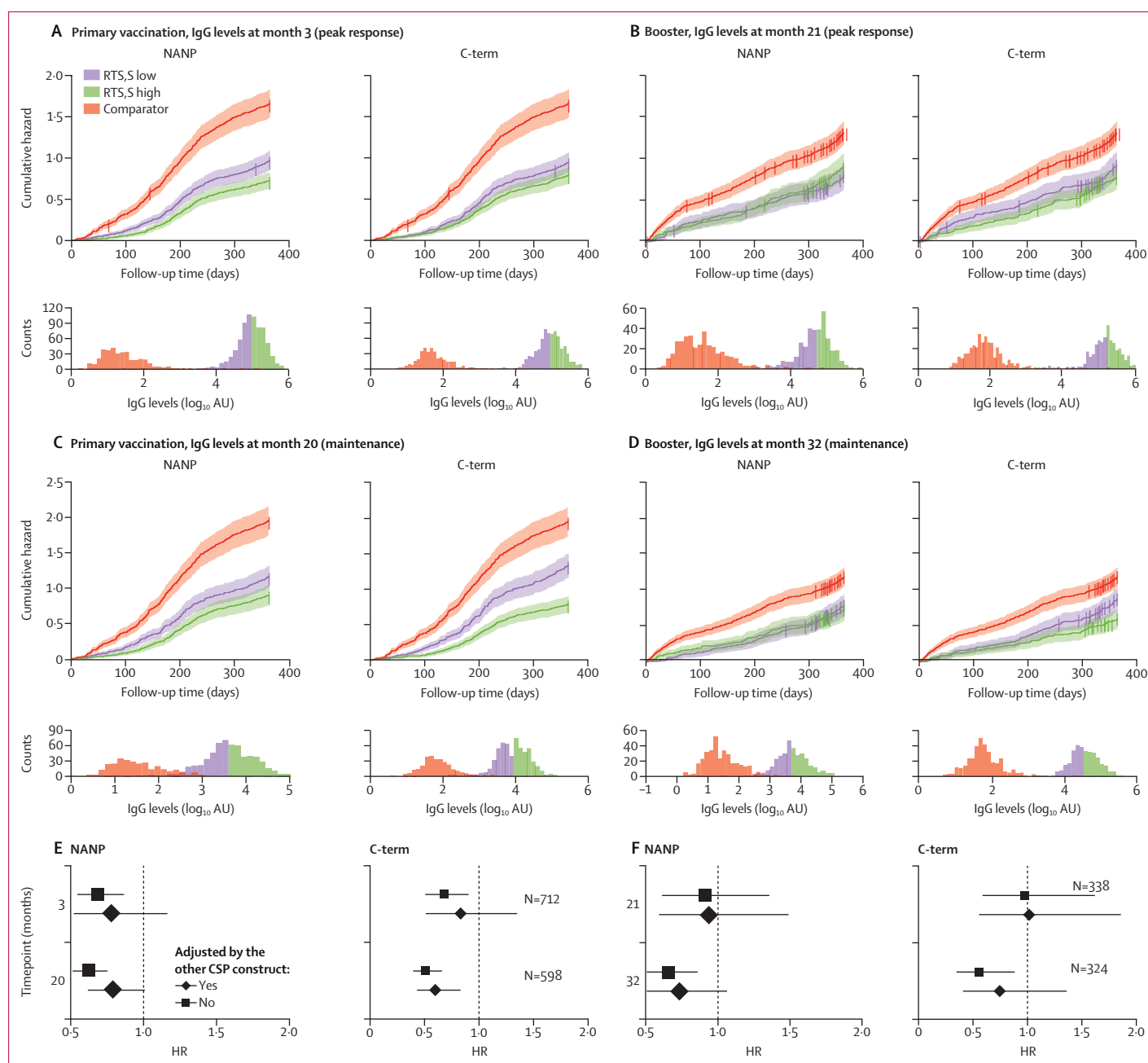


Figure 2: Association of IgG levels to NANP and C-terminal circumsporozoite protein with the hazard of *Plasmodium falciparum* clinical malaria episodes

(A–D) Cumulative hazard (malaria episodes per child) curves over 1 year after vaccination, estimated using a Nelson–Aalen estimator with inverse probability weighting to adjust for site. Histograms below each curve show the distribution of IgG levels in each group. (A) Primary vaccination: reference comparator (C3C, red) and vaccinated groups (R3R or R3C), the latter stratified by post-vaccination anti-NANP and anti-C-term IgG: high (≥ median, green) and low (< median, purple). (B) Booster vaccination: reference comparator (C3C, red) and boosted group (R3R), stratified as for part A. (C–D) Same as A and B, but with IgG levels sampled at later timepoints (months 20 and 32, respectively) as indicative of maintenance. (E, F) HRs: analyses are site-adjusted using stratified Cox regression. Diamond points indicate additional adjustment for IgG to the other circumsporozoite protein construct. The y-axis indicates sample timing: immediately post-vaccination (months 3 and 21; peak response) or months later (months 20 and 32; maintenance). AU=arbitrary units. CSP=circumsporozoite protein. C-term=C-terminal. HR=hazard change ratio per ten-fold IgG increase.

construct, none remained significantly independently associated with reduced risk (figure 2F). Since results derived from peak or maintenance IgG levels were not directly comparable because sample sizes and individuals included in each analysis differed slightly

due to sample availability, we performed a sensitivity analysis restricted to participants with complete data (ie, complete-case analysis) to assess the robustness of our findings. This analysis yielded consistent results with the main analysis, with no substantive changes in

the magnitude or significance of the associations (appendix 1 pp 17–23).

Finally, we generated predictions of vaccine efficacy across the observed range of IgG levels, including comparator vaccine recipients in the Cox models (figure 3). These curves can be interpreted as dose–response relationships. Steeper slopes indicated stronger correlation between IgG levels and risk reduction, capturing more heterogeneity in vaccine efficacy among vaccinated individuals. As expected, antibody maintenance levels (at months 20 and 32) could explain greater variability in vaccine efficacy compared with peak levels (months 3 and 21). This was due to mainly two reasons: (1) their curves had steeper slopes (ie, smaller HR per ten-fold increase in calibrated IgG levels; figure 2) and (2) IgG levels measured at these later timepoints had greater dispersion across the

population compared with peak IgG levels (appendix 1 p 15).

Discussion

The findings of this study show that circumsporozoite protein IgG levels waned between month 3 and month 20 at a significantly faster rate for NANP specificities over those targeting C-terminus, indicating distinct durability of antibody levels. The booster dose enhanced the C-terminal IgG levels, approximately doubling the peak geometric mean response after primary vaccination, contrary to the NANP IgG response, which was only half of that observed after primary vaccination. Importantly, measurement of the maintenance of IgG responses appeared to be more strongly associated with protection than IgG levels measured at peak. The differences between NANP and C-terminal antibody responses could

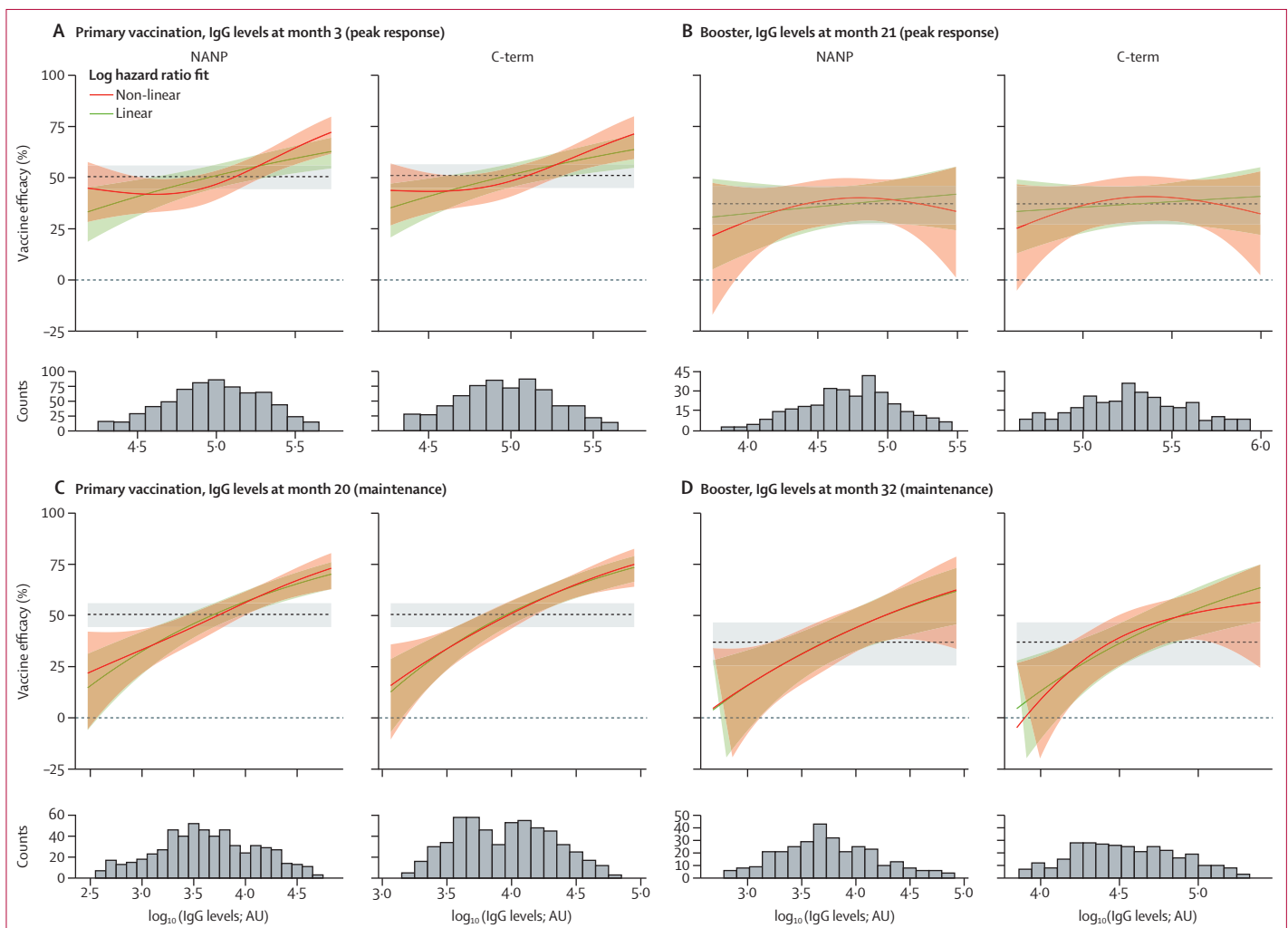


Figure 3: Predicted vaccine efficacy against multiple *Plasmodium falciparum* clinical malaria episodes over 1 year after vaccination

(A and C) Following primary doses. (B and D) Following booster doses. The green curve represents predictions from a linear log-hazard-log-IgG model, while the red curves show flexible non-linear spline fits (knots selected by AIC). Thick lines indicate point estimates; shaded ribbons represent 95% CIs. Each plot includes a horizontal dashed line and grey ribbon denoting overall vaccine efficacy, corresponding to predictions when IgG is unknown. All models were adjusted for site (via stratified Cox regression), age, and sex (as covariates in the outcome regression). AIC=Akaike Information Criterion. AU=arbitrary units. C-term=C-terminal.

be related to a strong B-cell activation due to the repetitive and disordered structure of the NANP major repeat, which can affect immunodominance, B-cell differentiation fate, and germinal centre selection.^{23,24} A key finding of this study was that IgG levels measured 18 months after primary vaccination and 12 months after booster were stronger correlates of protection than peak responses measured at 1 month after vaccination. We previously observed this in the RTS,S/AS02_A phase 2b trial,^{25,26} where maintenance of IgG levels had stronger predictive value than peak responses. RTS,S and R21, as with many other vaccines, induce a biphasic IgG decay, with an initial rapid loss of most antibodies (by ~90%, with a half-life of ~1.5 months) and a slower phase in which a smaller fraction is maintained (~10%, with a half-life of ~2 years).^{8,14,27} Consequently, around 6 months after re-vaccination, antibody levels would be expected to be dominated by the maintenance component rather than the transient peak response. However, further work is needed to empirically define the optimal timepoints for reliably capturing antibody maintenance and its relationship with long-term protection. This work could be investigated by repeated antibody surveillance in selected vaccinated subcohorts to inform programmatic decisions such as booster timing. Although peak IgG levels mainly reflect the early decay phase, antibody measurements at later timepoints might better capture sustained responses, which is relevant for long-term protection. Moreover, RTS,S-induced IgG responses are more homogeneous among individuals at peak response after primary vaccination.^{8,12} This homogeneity progressively diminishes over the following months as different antibody decay rates introduce a new source of variability, particularly for NANP.

Heterogeneity of circumsporozoite protein-specific antibody responses is a prominent feature of both RTS,S and R21 vaccination. Although few population-level predictors of immunogenicity were identified in this study or in previous ones,^{8,13,28} substantial individual-level differences clearly exist and could be driven by genetic factors, malaria history, and other previous and current exposures that shape the immune response.

NANP- and C-terminal-specific IgG levels are often strongly correlated,^{14,29} likely reflecting shared individual-level determinants of vaccine responsiveness and introducing potential confounding. In addition, although IgG responses to NANP and C-terminus are generally considered non-cross-reactive,^{30,31} low cross-reactivity might occur through NANP-like motifs present in the C-linker region,³² raising the possibility that apparent C-terminal-associated protection could partly reflect recognition of repeat epitopes. To account for this, we included models adjusted for IgG levels against the alternate circumsporozoite protein construct. After adjustment, the estimated protective effect was attenuated, and statistical significance often fell below our predefined significance threshold, although a

consistent trend remained. Taken together, these findings suggest that the association between C-terminal-specific IgG levels and protection, particularly after the booster dose, might not be fully explained by confounding through their correlation with NANP-specific IgG responses.

Observational analyses from this and other RTS,S trials have reported associations between stronger C-terminal-specific humoral responses and increased protection. In children from a phase 3 trial, C-terminal polymorphisms were associated with reduced vaccine efficacy.³³ We have also previously shown that higher avidity of anti-C-terminal IgG predicted protection independently of NANP-specific IgG responses.¹⁴ In a previous phase 2b trial, antibodies to the C-terminus were associated with functional Fc-dependent activity, which might contribute to protection against *P. falciparum*.⁹ By contrast, human monoclonal antibodies targeting C-terminus isolated from adults vaccinated with whole attenuated sporozoite vaccines showed poor binding to live sporozoites and minimal inhibitory or protective activity,³² which is consistent with low surface accessibility of the C-terminus on intact sporozoites. However, circumsporozoite protein undergoes a post-cleavage conformational change during hepatocyte invasion that exposes C-terminus, a stage at which C-terminal-specific antibodies are thought to be functionally more relevant than before reaching the liver.³⁴ Although C-terminus is less accessible than NANP, binding of repeat-specific antibodies might induce or stabilise conformational changes that enhance C-terminal exposure and enable Fc-mediated effector functions such as cytotoxicity or phagocytosis.³⁵ Nevertheless, evidence for synergistic activity between NANP- and C-terminal-specific antibodies remains insufficient, highlighting the need for further studies to clarify the functional relevance of C-terminal-targeting antibodies in vivo. Unlike the NANP repeats, the C-terminal region contains T-cell epitopes that induce cellular responses, which might contribute to protection.^{36–38} Therefore, the stronger association observed between C-terminal antibody responses and protection might reflect coordinated humoral and cellular immunity directed to this region.

Our results reinforce the idea that anti-circumsporozoite protein antibodies are not strong correlates of protection when measured at a single timepoint and capture heterogeneity in vaccine efficacy rather poorly. Between the lowest and highest observed IgG levels, we predicted differences in vaccine efficacy ranging from 25% to 75%. Although this range was a little larger using maintenance IgG levels, they preclude establishing meaningful protective thresholds. Moreover, relying on a single-timepoint peak response, as typically collected in clinical trials, will likely be inferior to using later single-timepoint measurements, which capture not only initial vaccine take and response but also maintenance mechanisms. This hypothesis is supported by our results, where

maintenance-phase anti-circumsporozoite protein IgG levels appear to be better predictors for two reasons: (1) IgG fold increases were associated with larger relative reductions of malaria hazard (ie, lower HR estimates) and (2) IgG levels had greater individual variability during the maintenance phase, likely because memory processes introduce additional heterogeneity. In short, prediction is better and the predictive biomarker range is wider with later single-timepoint measurements than with a single-timepoint peak measurement. This possibility should ideally be re-examined in studies where anti-circumsporozoite protein is measured repeatedly with sufficiently closely spaced sampling to assess antibody kinetics at an individual level, and determine whether instantaneous anti-circumsporozoite protein IgG might indeed emerge as a much stronger correlate of protection.

A limitation of this study is that some sample sets from children were incomplete, particularly in Lambaréné (Gabon), Bagamoyo (Tanzania), and Nanoro (Burkina Faso). Additionally, although we prioritised including individuals with the highest number of samples available, not all children had data for all timepoints. Consequently, the HR calculated using either peak-based or maintenance-based antibody levels included slightly different subsets of participants. Moreover, analyses based on IgG measurements obtained after the follow-up period (month 20 and month 32) necessarily included only participants who remained uncensored during the follow-up period (ie, who did not drop out or die). This dependency links the missingness of the predictor (IgG) to the censoring process, potentially introducing selection bias. However, dropouts or deaths were rare during the follow-up and the complete-case analysis showed similar results. Total IgG levels alone do not fully characterise the antibody response and, as we have previously reported in studies with smaller sample sizes, IgG subclasses and other isotypes have been associated with protection.¹³ Further longitudinal cohort studies with extended follow-up, incorporating other antibody isotypes and subclasses as well as integrated functional assessments, would be valuable to clarify the determinants of long-term protection and to define the relative contribution of C-terminal-specific antibodies within the broader immune response to RTS,S.

Our findings show that the RTS,S booster preferentially enhances C-terminal-specific IgG responses, while only modestly boosting NANP-specific antibodies. C-terminal IgGs were more durable than NANP responses after both primary and booster vaccination and correlated with protection against malaria. These associations were stronger for maintenance than peak IgG levels, indicating that antibody durability better predicts protection. These results emphasise the need to measure the antibodies against different vaccine antigen epitopes and their maintenance, to distinguish different immune

mechanisms involved in vaccine protection, and to guide optimisation of RTS,S composition, booster schedules, and the design of next-generation malaria vaccines.

Contributors

CDo, GM, CDa, BM, STA, HS, HT, BG, SO-A, KPA, PA, AN, and JW designed research studies. SD, ML-E, LS, MV, CJ, RM, DM-E, and AJ performed laboratory measurements. MM, HS, DD, STA, CJ, JW, LS, DM, ML-E, DM-E, GM, and CDo acquired the data. CDo, GM, LP, RA, SO-A, KPA, STA, HT, PA, AN, BM, CDa, and JW coordinated the studies. LS, RS and DM analysed the data. ML-E, LS, DM, GM, and CDo wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the final version. ML-E, LS, DM, GM, and CDo accessed and verified the data. All authors had full access to the data in the study and were responsible for the final decision to submit the paper for publication.

Equitable Partnership Declaration

The authors of this paper have submitted an Equitable Partnership Declaration (appendix 2). This statement allows researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This statement is part of The Lancet Group's broader goal to decolonise global health.

Declaration of interests

BM declares consulting fees from NobelPharma Co. (Japan) for advisory work on malaria vaccine development. All other authors declare no competing interests.

Data sharing

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Requests will be evaluated to ensure that the intended use is consistent with ethical requirements and the informed consent provided by all study participants. All shared data will be kept de-identified to protect participant confidentiality.

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See Online for appendix 2

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